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Combining insecticide treated bed nets and indoor residual spraying for malaria vector control in Africa

This thesis is submitted in fulfilment of a degree of Doctor of Philosophy at the
University of London, School of Tropical Medicine and Hygiene

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2012

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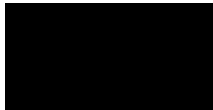
London School of Hygiene and Tropical Medicine

Declaration

I, Fredros Oketch Okumu, confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:

A solid black rectangular box used to redact the signature of the author.

Date.....18th April 2012

Abstract

Background: Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are the preferred techniques for malaria vector control in Africa, where their application has already contributed to significant reductions in the burden of the disease. Even though both methods are commonly used together in the same households, evidence of greater health benefits due to these combinations as opposed to use of either ITNs or IRS alone has been minimal and inconclusive.

Objectives and methods: The main aim of this research was therefore to contribute to this essential evidence, by way of experimental hut studies and mathematical simulations. I investigated whether there would be any added protective advantages when any of three selected long lasting insecticidal nets (LLINs) are combined with any of three selected IRS chemicals, as opposed to using any of the treatments alone. Data generated from the experimental hut studies was then input into an optimised deterministic mathematical model, simulating a typical malaria endemic village.

Results and conclusions: Both the field studies and the simulations showed that any synergies or redundancies resulting from LLIN/IRS combinations are primarily a function of modes of action of active ingredients used in the two interventions. Where LLINs are already present, addition of IRS would be redundant unless the IRS chemical is highly toxic, but where IRS is the pre-existing intervention, these combinations always confer improved protection. Therefore, IRS households should always be supplemented with nets, preferably LLINs, which not only protect house occupants against mosquito bites, but also kill additional mosquitoes. Finally, where resources are limited, priority should be given to providing everybody with LLINs and ensuring that these nets are consistently and appropriately used, rather than trying to implement both LLINs and IRS in the same community at the same time.

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I have ridden many waves created by many people who came before me. I have been taken to the hills and found a number of escape routes, through which we can lead our people away from the current public health challenges. Furthermore, I have been made to believe that this is not merely a dream, but a realistic opportunity to meet the health needs of all mankind. Yet, even as I finalize my PhD work, which I started exactly three years ago, I know that in the times ahead I will continue to ride many new and many old waves, created by many new and old friends. For this reason, I find it a particularly monstrous task to single out any one person, two people or even ten people to convey my direct appreciations to in this specific occasion. I must therefore only say thank you, so profoundly indeed, to all who have participated in one way or another in the ‘planning and construction’ of both ‘me and my career’ during the course of my PhD studies. In very specific terms however, the ink may never stop flowing if I do not use this same opportunity to salute the following extraordinary persons and institutions:

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The Ifakara Health Institute, Tanzania: I first joined the Ifakara Health Institute as a master degree student under the field supervision of Sarah Moore, during which time I wrote a thesis on 'Medium range olfactory responses of malaria vectors to synthetic mosquito attractants'. It was here that I found a voice and true recognition as a junior scientist. It was also here that, because of the people and the work environment, I began to recognize and appreciate the vastness of opportunities that we have in our worlds to tackle both current and future global health challenges. Here, I convinced myself that if you think, plan and focus, it is possible to shoot down a space-borne missile, using a hand

held gun loaded with just a single bullet. Though most of my postgraduate education has been through Universities outside Tanzania, most of my scientific research until now has been done here. Therefore, I must in very special terms express my heartfelt gratitude to the institute, and its management.

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one of the best managers of provincial malaria control programs in Ethiopia. When I later published my first ever research paper, it was actually based on work that I had done starting as a 19 year old high school graduate, under the keen guidance of Bart Knols and my old friend Dr. Ulrike Fillinger, a super-attentive scientist, who also worked in Bart's group at the time. Most important to say here is that without the opportunity that Bart created in Kenya during this special period, I never would have gotten that 'all important shot of life' at a career in scientific research. Thank you very much Dr. Knols. Your friendship and scientific guidance remains valuable today and ever.

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Volunteers participating in the experimental hut studies: The field studies that I conducted during my PhD studies required that human volunteers sleep inside the experimental huts at night, so that mosquitoes could be attracted into the huts and trapped. This is by far an extremely tedious task and requires an overly strong will and dedication. Moreover, in today's society where people mostly seek instant gratification, volunteering in an experimental hut study, for five nights a week for sometime up to six months continuously, takes a unique willingness to relinquish comfort for the sake of non-immediate benefits of research. I must therefore salute all of the nearly 35 volunteers who worked with us at different times during my studies, and who maintained a cheerful spirit at all times, even when the weather was not friendly. I wish them all the very best and remain hopeful that the research they have participated in, will yield the much needed health benefits to their communities and to the continent as a whole.

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Contributions by other researchers in co-authored papers presented in this thesis: This thesis is in the form of a collection of research papers. Given the nature of this research field, the work is almost always collaborative, and my own contribution as a PhD student was never in any case equivalent to 100% of the total input into any of the chapters included in this thesis. I would therefore like to also acknowledge the contributions of all my co-authors in the respective papers making up the core chapters of the thesis. More specific details of their involvement as co-authors can be found in the respective chapters or published research papers. The relevant specific co-author contributions were as follows:

1. Chapter II (estimated percentage of my own contribution $\approx 90\%$): The work included in this chapter was co-authored by me and my supervisor. Even though I conducted the actual review and drafted the original manuscript, both my supervisor and I verified the gathered information and edited the manuscript before it was eventually published.
2. Chapter III (estimated percentage of my own contribution $\approx 80\%$): The work described in this chapter was co-authored by me as the first author, my supervisor as the last author, and six other researchers as middle authors. All the experiments were designed by me, my supervisor, Dr. Sarah Moore and Dr. Tanya Russell. I analysed the data under supervision of Dr. Moore and then drafted the original

manuscript for publication. My supervisor and I then edited the manuscript before submitting it to the journal. Jason Moore, Tanya Russell, and my supervisor were involved in the original designs of the experimental huts. Jason Moore was the lead engineer who constructed the huts and all its accessories including the traps and baffles. Finally, Edgar Mbeyela, Mark Sherlock, Robert Sangusangu, and Godfrey Ligamba supervised various field experiments described in this chapter.

3. Chapter IV (estimated percentage of my own contribution $\approx 80\%$): This chapter was co-authored by me (first author), my supervisor (last author) and 7 other authors. Individual contributions were as follows: all the experiments were designed by me with the assistance and supervision of Dr. Sarah Moore. Statistical training and support was provided to me chiefly by Dr. Lena Lorenz, but also by Dr. Liz Turner and Prof. Mike Kenwood, both at the LSHTM. I drafted the original manuscript, after which my supervisor and I edited it into a journal ready format. Edgar Mbeyela, Godfrey Ligamba assisted with the supervision of the field experiments. Jason Moore was the lead engineer in charge of field logistics, hut construction and maintenance. Finally, Dr. Beatrice Sumaye, conducted all the molecular analysis of mosquitoes, as reported in the chapter.
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6. Chapter VII (estimated percentage of my own contribution $\approx 40\%$): This was also a co-authored chapter. However in this case, the research paper was not primarily my own work, but Dr. Gerry Killeen's. My participation was that I provided the basic modelling equations (from my work in Chapter VI) and participated in its improvements before new simulations could be done. I also acted as the last author of the published manuscript, ensuring consistency and authenticity of both the parameter values and the equations. Dr. Sarah J. Moore (my supervisor), and Dr. Nakul Chitnis participated by ensuring that the annotations and the parameter extractions were appropriately conducted. Dr. Killeen drafted the first manuscript,

after which himself and I participated in writing the final version of the published manuscript.

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List of abbreviations

| | Abbreviation | Meaning |
|----|---------------------|---|
| 1 | ACT | Artemisinin-based Combination Treatment |
| 2 | BMGF | Bill and Melinda Gates Foundation |
| 3 | CDC | Centres for Disease Control |
| 4 | CDC-LT | Centres for Disease Control- Light Traps |
| 5 | DDT | Dicloro-diphenyl-tricloroethane |
| 6 | DNA | De-oxyribonucleic acid |
| 7 | EIR | Entomologic Inoculation Rate |
| 8 | GLM | Generalized Linear Model |
| 9 | GLMM | Generalised Linear Mixed Model |
| 10 | GMAP | Global Malaria Action Plan |
| 11 | HIV | Human Immune-deficiency Virus |
| 12 | ICIPE | International Centre of Insect Physiology and Ecology |
| 13 | IHI | Ifakara Health Institute |
| 14 | IPT | Intermittent Preventive Treatment |
| 15 | IRS | Indoor Residual insecticide Spraying |
| 16 | ITN | Insecticide Treated Nets |
| 17 | IVCC | Innovative Vector Control Consortium |
| 18 | IQR | Inter-quartile Range |
| 19 | kdr | Knock-Down Resistance |
| 20 | LLIN | Long Lasting Insecticide Treated Nets |
| 21 | LLUN | Long Lasting Untreated Nets |
| 22 | LSHTM | London School of Hygiene and Tropical Medicine |

| | | |
|----|--------|---|
| 23 | malERA | Malaria Eradication Research Agenda |
| 24 | NIMR | National Institute Of Medical Research, Tanzania |
| 25 | PE | Polyethylene |
| 26 | PCR | Polymerase Chain Reaction |
| 27 | PMD | Para-methane 3,8 diol |
| 28 | PMI | US' Presidents Malaria Initiative |
| 29 | RBM | Roll Back Malaria |
| 30 | RCT | Randomised Control Trials |
| 31 | TB | Tuberculosis |
| 32 | TPP | Target Product Profiles |
| 33 | TPRI | Tanzania Pesticide Research Institute |
| 34 | USAID | United States Agency for International Development |
| 35 | WHO | World Health Organization |
| 36 | WHOPES | World Health Organization Pesticide Evaluation Scheme |

PART ONE

Preview of Part One

This part of the thesis consists of two chapters:

Chapter I: General Introduction. This chapter describes the overall burden of malaria in Africa, the current efforts to control it and an overview of challenges facing malaria vector control today. A general overview of the PhD study, a statement describing the research problem and the main research objectives are listed at the end of the chapter.

Chapter II: Indepth Review. This chapter contains an indepth review on the main subject of this PhD study, i.e. potential benefits of combining insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) for malaria control in Africa. The chapter also presents an analysis of the modes of actions of common insecticides used for ITNs and IRS, as well as key research questions that should be focused on to generate the necessary evidence needed to support decision making regarding ITN/IRS combinations.

Chapter I

General Introduction

Overview of malaria in Africa

Since 2005, the World Health Organization (WHO) has presented a progressively improving picture of the malaria burden in Africa [1-5]. With reference to goals established at the African presidents' summit on malaria in Abuja in 2000 [6], and subsequent resolutions outlined in the Roll Back Malaria Global Strategic Plan 2005-2015 [7], it is evident that significant progress has been made in the last decade. In the same period a number of important lessons have been learned that will enable future international collaboration towards the renewed interest in elimination and perhaps eventual eradication of malaria. Endemic countries and the global community are scaling-up the use of effective interventions, and malaria burden in Africa and elsewhere around the world is generally declining [1-4]. Nevertheless, the situation is not entirely positive and despite all the above assertions, the long-established description of malaria as one of the world's most devastating human diseases remains undeniably accurate.

Some 3.2 billion people worldwide still live in areas at risk of malaria and according to the latest world malaria report, there were at least 255 million cases of the disease (resulting in nearly 800,000 deaths) in the year 2009 [3]. The geographical distribution of malaria [8] and its impacts on public health systems around the world (especially in low income tropical countries) make it the most significant human infection besides the human immunodeficiency virus (HIV), diarrhoeal diseases,

pulmonary tuberculosis (TB) and other respiratory tract infections [9]. Moreover, the disease has an inexplicably complex relationship with poverty in most endemic communities in Africa. While poverty sustains conditions where malaria thrives, malaria also impedes economic growth and keeps communities in poverty [10]. Today, nearly 90% of all malaria cases and about 75% of all deaths occur in sub-Saharan Africa [3], where other than the high mortality and morbidity, economic burden of the disease is also enormous; including up to 1.3 % reduction on economic growth [11].

Current best practices for tackling malaria include: 1) prompt diagnosis (using light microscopy or rapid diagnostic tests) followed by treatment with effective medicines (such as artemisinin based combination therapy (ACTs), 2) vector control (including primarily the use of insecticide treated bed nets (ITNs) and indoor house spraying with residual insecticides (IRS)) and 3) intermittent preventive treatment (IPT) of pregnant women, infants or children [3]. Under the current Global Malaria Action Plan [12], public health authorities can aim at sustained universal coverage with these existing malaria prevention and control measures. But because of well-known efficacy and cost-effectiveness, vector control through ITNs and IRS, and use of ACTs, have inevitably become the most dominant malaria interventions, enjoying incomparable political will and user acceptance rates. Regrettably, there is not yet any effective vaccine for malaria prevention [13], despite several recent breakthroughs [14-16], which indicate likelihood of an effective vaccine in the near future.

Historical trends of malaria vector control: the rise of IRS and ITNs

Until mid 1940s, control of mosquitoes and malaria depended upon environmental management, improved housing, improved sanitation, biological control, and use of

toxic larvicides [17-24]. Nets, whether insecticidal or non-insecticidal, and house spraying with residual insecticides were largely unknown at that time; even though nets may have been used in ancient times by certain isolated communities around the world, for purposes including but not limited to mosquito bite prevention [25].

Methods of controlling malaria vectors changed dramatically during World War II, when insecticide-based methods were first used in large scale against adult mosquitoes. Appreciably, the most significant event at the time was the introduction of DDT (dicloro-diphenyl-tricloroethane), which quickly become the main weapon against malaria [26, 27]. It was also around this period when bed nets were first treated with insecticides, not surprisingly DDT, to protect soldiers fighting in the tropics from leishmaniasis and malaria [28]. Unfortunately, due to the high levels of effectiveness observed, house spraying with DDT dominated malaria control so much so that research and application of other vector control methods or insecticides rapidly declined. The ITN technology for example would remain shelved and forgotten for nearly four decades.

Between 1955 and 1969, WHO led the first Global Malaria Eradication campaign, which was dependent mainly on vector control through periodic spraying of DDT in houses [29]. This was the first global advocacy for IRS in malaria control even though the African continent was hardly covered [30]. Though the intended global eradication was not achieved, malaria risk was purged from millions of people, in Europe, North America and most of the Caribbean, Latin America, Asia and the Middle East [17, 26, 29, 30]. But hardly a decade after the program was launched, challenges such as insecticide resistance, controversies about environmental impacts of DDT, donor fatigue and operational difficulties became serious concerns [17, 26, 27, 31-33], and eventually in 1969, this global campaign was halted.

Later in 1985, IRS was officially deemphasized and malaria control was decentralized to be managed under national primary health care programs [34]. The intervention was continued in only a small number of countries such as Eritrea, Ethiopia and Madagascar and Latin American countries like Brazil, Colombia, Ecuador and Venezuela, where DDT remained the insecticide of choice [26, 35]. A small number of countries in the southern Africa region namely Namibia, Mozambique, Botswana, South Africa, Swaziland which had promoted IRS actively since 1930s also continued implementing the strategy [1, 36, 37]. As the support for DDT was fading, several alternative insecticides were tested against malaria vectors [38]. These included chlorinated hydrocarbons such as dieldrin [39] and organophosphates like dichlorvos [40], fenitrothion [41] and malathion [42] among others. These efforts were aimed at finding alternatives to DDT which would have no negative environmental impacts and no mammalian toxicity but to which target vectors would remain susceptible. Later, synthetic pyrethroids such as deltamethrin and lambda cyhalothrin [43-45] were also tested. But none of these would eventually get to be used as widely as DDT had been.

When in the early 1990s, public health emphasis was beginning to shift back towards prevention, ITNs re-entered malaria control strategies [46]. Evidence that insecticidal nets reduce malaria related mortality or morbidity had begun to appear [47, 48], and support for ITNs gradually increased. The Roll Back Malaria program was launched in 1998 and has since then, advocated for intensified use of ITNs. WHO also recommended IRS, including application of DDT as long as user countries adhered to recommendations of the Stockholm Convention on Persistent Organic Pollutants [49, 50]. In 2000, malaria control targets including coverage with ITN and IRS were set by African heads of states to 60% of at-risk populations [6]. These were

revised in 2005 to 80% under the RBM strategic plan for 2005 to 2015 [7, 51] . Lately the targets have again been shifted to universal coverage as recommended in the current Global Malaria Action Plan, championed by the WHO/RBM partnership [12].

Recent trends of malaria control using IRS and ITNs

Analyses of ITN and IRS use in recent years reveals two especially encouraging trends. The first is the increasing acquisition of long lasting insecticide nets (LLINs) as opposed to ordinary ITNs, and the second is the gradual increase in the coverage of both ITNs and IRS in malaria endemic countries.

Gradual change from using ordinary ITNs to the use of LLINs

Some 10-15 years ago, nets used against malaria mosquitoes were mainly non-insecticidal [52, 53]. These untreated nets (as they are now generally known) work mainly as physical barriers preventing mosquito bites when people are asleep under them. They can provide modest protection when used properly and when in good condition [54-57], but their effects rapidly deteriorate when improperly used and when they are torn, in which case mosquitoes can still enter and bite the occupants [58], rendering the nets nearly useless. The concept of insecticide treated nets was considered as a way to extend the protective efficacy of nets and to induce community benefits to not only users but also non users [59, 60]. Towards the end of 1990s, net treatment and re-treatment with effective insecticides intensified, and new nets were now increasingly being factory treated, or sold untreated but bundled together with insecticide kits. The actual process of net treatment at the community level was in itself operationally very difficult to sustain and the practice quickly become a major impediment to the ITN strategy in general [56]. Without regular re-treatment, the

hand treated ITNs quickly reverted to the state of ‘untreated nets’ as their insecticidal efficacy quickly declined due to natural decay of the insecticides or attrition from repeated washing [61, 62].

New technologies of net manufacturing utilize long lasting fibres and more permanent impregnation techniques to produce long lasting insecticidal nets (LLINs) [63, 64]. The insecticide is either incorporated within the fibres or coated on the fibre surfaces using resins. According to WHO guidelines for approval, an LLIN must retain effective biological activity, killing mosquitoes without re-treatment for at least 20 washes and three years of use [65]. In practice however, these nets are reported to actually last between 3-5 years, and in some instances they have been shown to remain effective even after 7 years of use [64, 66-68].

Certain LLINs also have a regenerative property, meaning that their insecticidal activity can be boosted in the process of their use [69]. For example when used Olyset® nets are washed and heated, the active ingredient embedded inside the fibres becomes exposed onto the fibre surfaces thereby rejuvenating the desired toxicity of these nets to mosquitoes [64, 69]. Because of their superior insecticidal properties, robust nature and extended half-life, LLINs provide greater and more sustainable protection than ordinary hand treated ITNs. In fact it has been projected that with these long lasting net formats, only modest coverage is required to provide desired communal protection against malaria transmission [70] .

Until now, WHO has approved seven different LLIN brands (Table 1) and six different insecticides for treating nets [71]. There is however an obvious preference by both the international community and the malaria endemic countries for LLINs as opposed to ordinary ITNs. Data collected by WHO between 2000 and 2009 [1-3], shows very clearly that distribution and sale of nets has been gradually shifting from

ordinary ITNs to LLINs. As early as 2005, the supply chain of nets delivered to Africa, Europe, the Americas and Eastern Mediterranean, already consisted of more LLINs than ordinary hand treated ITNs. Moreover, based on the guidelines put forward in the current WHO/RBM global malaria action plan [12], and because of the improved cooperate responsibility of businesses and industrial partners, it is very likely that only LLINs will be produced and distributed in coming years.

Table 1: WHO-approved and recommended long lasting insecticide treated nets (LLINs)

| LLIN | Manufacturer | Active ingredient (a.i) |
|------------------------------|--|--|
| Durane [®] | Clarke Mosquito Control, USA | Alpha cypermethrin incorporated into polyethylene (270mg a.i /m ²) |
| NetProtect [®] | Best Net Europe ltd, United Kingdom | Deltamethrin incorporated into polyethylene (68-79mg a.i /m ²) |
| Interceptor Net [®] | BASF ltd-The Chemical Company, Germany | Alpha cypermethrin coated on polyester (200mg a.i /m ²) |
| PermaNet 2.5 [®] | Vastegaard Frandsen, Denmark | Deltamethrin coated on polyester with strengthened border |
| PermaNet 3.0 [®] | Vastegaard Frandsen, Denmark | Combination of deltamethrin (118 mg a.i /m ²) coated on polyester with strengthened border for side panels and deltamethrin (180 mg a.i /m ²) and pyperonyl butoxide (synergist) incorporated into polyethylene for the roof |
| PermaNet 2.0 [®] | Vastegaard Frandsen, Denmark | Deltamethrin coated on polyester (55-62mg a.i /m ²) |
| Olyset [®] | Sumitomo Chemicals ltd, Japan and A to Z ltd, Tanzania | Permethrin incorporated into polyethylene (1000 a.i /m ²) |

Gradual increase in coverage with both ITNs and IRS

Even with widespread incompleteness of reporting, WHO-collated data, government reports and independent evaluations all show large increase in ITN and IRS coverage. An increasing number of countries are approaching or reaching the previous and present malaria control targets [3, 6, 7, 12, 51]. Already, between 2004 and 2007, more than 127 million nets were distributed freely or at subsidized costs to people living in malaria risk areas and about 96 million of these nets went to Africa [1]. In addition some 41 million households were sprayed with residual insecticides. Just three years later, new estimates suggest that approximately 289 million nets would have been delivered to sub-Saharan Africa by the end of 2010, matching the needs of at least 76% of the 765 million vulnerable people in the region [3].

In sub-Saharan Africa, utilization of ITNs increased exponentially subsequent to the Abuja declaration in 2000 [6]. Between 2000 and 2003, the increase was marginal and coverage of nets, treated or untreated remained dismal [52, 53, 72]. For children under five years, untreated nets may have reached 20% in few countries (e.g. Guinea-Bissau, Mali, Sao Tome and Principe, The Gambia, Comoros, Tanzania, Chad and Benin), but coverage with ITNs remained below 5% in nearly all sub-Saharan African countries [52]. Only the islands of Sao Tome and Principe, and The Gambia reported ITN coverage of greater than 10% among under-five year olds. Monasch *et al.*, 2004 estimated that based on 1998-2002 health surveys, coverage in Africa with 'any nets' was 15%, but that ITN-specific coverage was only 2% [72]. Fortunately by this time, nearly all malaria endemic countries in Africa had adopted ITNs or LLINs into malaria control policies [53].

By 2004, good progress was being made as public health authorities revitalized efforts towards health equity; and as novel delivery methods for ITNs such

as social marketing and mass distribution became popular [73-76]. In Malawi there was 8% coverage with any net in 2000 but this had risen to 36% coverage with ITNs by 2004 [77]. Between 2003 and 2004, ITN use among children under-five years increased from 4.6 to 23% in Senegal, 10.2 to 16% in Tanzania and 6.5 to 23% in Zambia [53]. Other notable success stories were Togo and Niger where house hold level ITN possession rose from 8 to 63% and 6 to 61% respectively [52, 53]. Perhaps the best achievement at the time was Eritrea, which reached 63% ITN coverage by end of 2004 [78]. By 2007, when the new WHO targets were already in place [7, 51], countries reaching 60% household coverage now also included Kenya, Niger, Sao Tome and Ethiopia. [1]. Another terrific example has been Zambia where the latest Malaria Indicator survey has shown that since 2006, the proportion of households owning at least one ITN had risen by 38%, reaching 62% in 2008 [79]. The general continent wide coverage remained very low given that there were still extensive areas with large populations, including Congo, Sudan and Chad, where nets had not adequately penetrated [2]. Nevertheless, this situation has since dramatically improved. According to the latest WHO report, approximately 42% of households in Africa owned at least one ITN in mid-2010 and that 35% of all children under the age of five slept under ITNs [3]. Given that these coverage rates are still far below the targeted goals, and because some of the nets earlier delivered are now due for replacement, the scale up of LLINs still needs to be reinvigorated.

Coverage with IRS has improved significantly during the same period. The number of protected people in sub-Saharan Africa, which was estimated at 13 million in 2005 [4] had increased to about 75 million in 2009 [3]. Even though this figure may represent coverage of only about 10% of the total population of people at risk of malaria, the coverage of households actually targeted by IRS has been consistently

high in individual countries. Indeed it appears that more countries have met the IRS targets than ITNs targets. Mozambique, Swaziland and South Africa have been implementing joint regional IRS activities since 2000 and have witnessed a sustained suppression of malaria burden in the region [80]. Together with other southern Africa countries like Namibia, South Africa and Swaziland, they have consistently been attaining universal coverage in IRS designated areas in recent years [1, 80]. Other examples include Botswana where IRS consistently covered greater than 60% of risk populations between 2004 and 2007 [1], and Zambia, where IRS began in 2001 and where 40% of households targeted for IRS, were covered in 2008 [79].

Between the time of the DDT prohibitions in 1970s and the time when pyrethroids entered malaria control in 1980s and 1990s, only a handful of countries had continued IRS, often with excellent gains [1, 26, 35-37]. Today however, nearly two-thirds of countries in sub-Saharan Africa use IRS and WHO has approved 12 different insecticides for this purpose (Table 2) [81]. While some countries such as South Africa, have been alternating between DDT and synthetic pyrethroids (mainly to control insecticide resistance), the pyrethroids are generally favoured, arguable because they have lower mammalian toxicity, are more readily available, are applied in lower doses (making them more economically viable especially in areas where insecticides are shipped by road), and because of national and international restrictions surrounding DDT use.

Table 2: WHO-approved and recommended insecticides used for Indoor Residual Spraying (IRS) ^T

| Insecticide compounds | Formulations | Class group | Dosage (g a.i./m²) | Mode of action | Duration of effective action |
|------------------------------|--|--------------------|--------------------------------------|-----------------------|-------------------------------------|
| DDT | Wettable powder (WP) | Organochloride | 1-2 | contact | >6 months |
| Malathion | Wettable powder (WP) | Organophosphate | 2 | contact | 2-3 months |
| Fenitrothion | Wettable powder (WP) | Organophosphate | 2 | contact & airborne | 3-6 months |
| Pirimiphos-methyl | Wettable powder (WP) & emulsifiable concentrate (EC) | Organophosphate | 1-2 | contact & airborne | 2-3 months |
| Bendiocarb | Wettable powder (WP) | Carbamate | 0.1-0.4 | contact & airborne | 2-6 months |
| Propoxur | Wettable powder (WP) | Carbamate | 1-2 | contact & airborne | 3-6 months |
| Alpha-cypermethrin | Wettable powder (WP) & suspension concentrate (SC) | Pyrethroid | 0.02-0.03 | contact | 4-6 months |
| Bifenthrin | Wettable powder (WP) | Pyrethroid | 0.025-0.05 | contact | 3-6 months |
| Cyfluthrin | Wettable powder (WP) | Pyrethroid | 0.02-0.05 | contact | 3-6 months |
| Deltamethrin | Wettable powder (WP) and water dispersible granules (WG) | Pyrethroid | 0.02-0.025 | contact | 3-6 months |
| Etofenprox | Wettable powder (WP) | Pyrethroid | 0.1-0.3 | contact | 3-6 months |
| Lambda-cyhalothrin | Wettable powder (WP) and capsule suspension (WP) | Pyrethroid | 0.02-0.03 | contact | 3-6 months |

^T Table adapted from WHO evaluation homepage <http://www.who.int/whopes/quality/en/> [81].

Some major challenges facing the IRS and LLIN strategies

Insecticide resistance

Reduced susceptibility of mosquitoes to commonly used insecticides is arguably the number one challenge currently facing malaria vector control [82-86]. It is incriminated as having been the major cause of failure of the past Global Malaria Eradication Campaign [31, 32, 87]. Tables 1 and 2 above, show that there are only 4 classes of insecticides that are currently approved for IRS use, and that all the permitted insecticidal nets are based on just a single class of chemicals (i.e. synthetic pyrethroids). This situation, coupled with the possibility of cross-resistance between different insecticides [88, 89], illustrates the fragility of IRS and LLINs, which both insecticide-based vector control interventions, and emphasizes the urgent need for action. Moreover, the risk of target vectors developing resistance against various insecticides is greatly increased when a given insecticide is continuously used for long periods of time, without any measures aimed at delaying of managing resistance [90].

Physiological resistance of mosquitoes to insecticides can occur primarily in two ways. The first is through target site insensitivity, where the insecticide can no longer bind onto the target receptors in the mosquitoes. The most common and best described target site resistance is the *kdr* (knock-down resistance) mechanism, which occurs when there is a mutation in the genetic region coding for the sodium channels through which some organochlorines and pyrethroids are transported across insect cellular membranes. This causes physical alterations in structure, which is sometimes accompanied by a reduction in the overall number of these channels, so that the entry of insecticides into nerve cells is inhibited. It is this target site resistance mechanism which is also responsible for cross resistance between pyrethroids and organochlorides such as DDT [88, 91]. Target site insensitivity can also affect

insecticidal activity of other compounds such as organophosphates and carbamates, for example when there are alterations at the active site of the enzyme, acetylcholinesterase, thereby inhibiting binding of these insecticide groups. The other resistance mechanism results from increased metabolism and is characterised by either high levels or increased activity of enzymes (esterases, oxidases, glutathione-s-transferase), which are normally required by insects to detoxify chemicals including the insecticides. These particular mechanisms can act against multiple insecticide classes [88]. For example, elevated esterases have been associated with carbamates and organophosphates resistance, mono-oxygenases are involved in rapid metabolism of pyrethroids and also detoxification of some organophosphorous insecticides and glutathione-s-transferases are involved in detoxification of DDT [90].

Even though the precise extent of insecticide resistance in Africa is not yet clearly defined [92], there is a constantly growing evidence of its occurrence in the West Africa, Central Africa, East Africa, and also southern African countries [85, 86, 91]. To avoid exacerbating the problem of resistance, the type of insecticide used for IRS has either had to be changed in many countries e.g. Southern Africa [36], Bioko Island in Equatorial Guinea [93] and Mozambique [94] or it is being considered for change e.g. in Zanzibar (Dr. Peter McElroy, Pers Comm). Indeed, significant successes against malaria have been achieved with this strategy, one recent case being the change from pyrethroids to bendiocarb (a carbamate), for IRS in Benin, where there is a very high frequency of pyrethroid resistance among malaria vectors [95]. Today the international community, through major non-governmental initiatives such as the innovative vector control consortium (IVCC) have embarked on major initiatives to find alternative insecticides that can continue to perform even in areas where large proportions of disease-carrying mosquitoes are resistant to existing

insecticides [82]. It is therefore a matter of absolute importance to slow the onset and spread of resistance even as these new products are developed.

Human and vector behaviour

The second important challenge facing IRS and ITN strategies is the increasing overlap between human activity and mosquito activity, especially with regard to being inside or outside houses. Both ITNs and IRS are insecticide-based intra-domiciliary interventions. Primarily, these interventions target only those mosquitoes that enter and those that attempt to enter human houses. However, there is growing evidence that these particular tools cannot control all the malaria transmission that occurs in nature and that there is a significant residual proportion of transmission that continues to occur either outdoors or indoors at times when people are not yet under their bed nets [96-99]. There is also evidence that some specific groups of people spend a long time outside their houses and that these people are more at risk given that they do not benefit directly from the effects of the ITNs and IRS [99].

A few years ago, Killeen *et al* estimated that even in areas dominated by the indoor feeding anthropophagic malaria vectors, *An. gambiae sensu lato*, about 10% of malaria transmission was already occurring when people were outside their houses and effectively not using their nets [96]. Recent mathematical simulations by Govella *et al* have also now shown that in situations such as urban Dar es Salaam, Tanzania, outdoor malaria transmission may constitute as much as 50% of the overall transmission by vectors such as *Anopheles arabiensis*, a member of the *An. gambiae* species complex, which can readily feed outdoors and on non-human hosts in response to extended bed net coverage [99]. Thus the only benefit that people obtain outside the direct spectrum of IRS and ITN coverage is the indirect protection from

the communal effects of the interventions, such as that which results from the mass killing effects of the nets [60, 70].

Recent evidence now shows a dramatic shift in proportional composition of major malaria vectors. For example in Kenya and in Tanzania, the previously predominant *An. gambiae sensu stricto* has now been overtaken by *An. arabiensis* as the new dominant vector [100, 101]. The latter vector species may be anthropophilic (preferring to feed on humans over other vertebrates) and endophagic (preferring to feed indoors than outdoors) [102-104], but it is also known to very readily bite non-human hosts (e.g. cattle, where available), and also to more readily bite outdoors than *An. gambiae s.s* [103, 105-109]. These behaviours greatly lower the thresholds at which current intradomiciliary interventions like ITNs and IRS cease to be effective in areas experiencing this shift in mosquito populations [97, 110]. Besides, there are other species such as *An. coustani*, which are of minor importance as malaria vectors [111], and cryptic subgroups of *An. gambiae*, which are emerging as possible malaria vectors [112], which have very different behaviours from the common vectors, but which will have to be targeted as well if malaria elimination is to be achieved [84]. Fortunately, the public health research and donor communities are already pushing ahead towards development of new interventions that target mosquitoes in areas other than inside human houses [84].

The slow pace of development of new malaria control tools

Many malaria scholars will recognise that the excessive focus on vector control through IRS during the first global eradication campaign, and later chemotherapy through the primary health care units, resulted in an unprecedented slow-down in the pace of development of new agents for malaria control [31, 32]. There was very little

research conducted on new malaria control tools and strategies, meaning that effective malaria control tools remain a major limitation in public health.

The malaria eradication research agenda (malERA) consultative forums identified four key components for successful vector control [84]. One of these was the need for synergistic or complementary interventions that are applied through rationally designed programs in temporal or spatial combinations (Table 3). Besides, the current global malaria action plan recognizes that even though IRS and ITNs should be promoted as the key interventions against malaria, there should be an attempt to develop and evaluate new interventions that could then be applied at national or district level, based on local evidence from specific areas [12]. As shown in Table 3, this new global agenda for research towards sustained malaria control and malaria eradication extensively embraces the need to consider insecticides, insecticide formulations or new vector control methods that can circumvent the problems of resistance among vector populations, changing human and vector behaviour, and the inability of existing intervention methods to target the full spectrum of malaria transmission indoors and outdoors [84].

Table 3: Vector control focus points and tools required for sustained control and for eradication of malaria[@].

| Sustained control | Eradication |
|---|---|
| Effective insecticides for LLINs and/or IRS | Better vector monitoring and evaluation information to target interventions |
| Resistance monitoring and management | Effective insecticides for LLINs and/or IRS |
| Vector identification and incrimination | Resistance monitoring and management |
| Appropriate integrated vector management | Vector identification and incrimination |
| | Appropriate integrated vector management |
| | Targeted interventions for outdoor biting and resting mosquitoes |
| | Novel approaches to reduce permanently the high vectorial capacity of major vectors (e.g. genetic modification) |
| | Effective consumer products for vector control |

[@]Table adapted from The malERA consultative group on vector Control [84]

Preserving and optimizing effectiveness of ITNs and IRS

The dominant school of thought states that the best ways to preserve current effectiveness of existing primary vector control methods, LLINs and IRS, are measures that aim at preventing insecticide resistance among mosquito populations, for example development of new and alternative insecticides [82, 84]. While insecticide resistance is indisputably one of the most significant challenges facing malaria vector control today, malaria elimination and eventual eradication will also require combination of current best practices [98], leading to greater impacts than in situations where these tools are implemented singly. Where necessary, these combinations of existing interventions, e.g. LLINs and IRS, LLINs and larvicides, LLINs, LLINs and mosquito traps or house screening can first be simulated and their potential benefits explored using mathematical models before a selected set is experimentally tested or implemented in real life situations [98, 110].

Even as the world seeks additional and alternative tools to complement IRS and LLINs, these two methods themselves remain the most preferred [3, 12]. Moreover, existing evidence suggests that high coverage of households with LLINs and IRS are presently the most effective options available to control malaria in high transmission areas [98, 113, 114]. Their application must therefore be optimised through evidence based decision making processes, not only to preserve the accrued benefits, but also to ensure cost effectiveness of the strategies, especially when they are used together in the same communities

This thesis deals with one possible technique for preserving and optimizing effectiveness of existing tools, i.e. the combination of LLINs with IRS in the same households. The research was generally aimed at determining whether indeed such combinations would have advantages or disadvantages, relative to using either LLINs

alone or IRS alone, and therefore to provide a basis for decision making on aspects such as: 1) whether that strategy is necessary, 2) which insecticides are the most appropriate to be combined and 3) whether the strategy be cost-effective.

Overview of the PhD Research

Background and rational

ITNs and IRS are the most preferred techniques for malaria vector control [115-117]. Their application has led to reduced malaria burden in many endemic countries [3]. The two methods are commonly used together and many governments have incorporated both of them in state policies.

Any policy-based combinations of vector control methods require scientific verification for expected added value. This would enable policy makers to select the most appropriate combinations, for example IRS insecticides and types of ITNs, while considering factors such as baseline transmission intensities and the behaviour of the local vector populations. In situations where resources are limited, such evidence may also guide resource allocation. For example if it were determined that there is no added value from using IRS alongside ITNs, resources could be diverted to other sectors or strengthen existing ITN operations.

Today, most of the existing information on benefits of ITNs and IRS is derived from controlled trials where the methods were tested individually. However in operational programs, it is more common that the two methods are used together; either concurrently or one after the other. For example, IRS is often performed in response to malaria epidemics while ITNs are continuously distributed through national programs or public-private partnerships [118], resulting in a situation of

overlap between IRS and ITN coverage. Unfortunately though, there is not yet any substantive evidence of benefits or failures due to such combined use, or whether the two methods complement or diminish the beneficial effects of each other [119]. The other challenge is the determination of appropriate insecticides to be used where such combination is done. These and other important questions require controlled field experiments, conducted in malaria endemic areas, where vectors are monitored under exposure to different IRS compounds, ITNs or combinations thereof.

I proposed to conduct field studies to determine the behavioural and toxicological effects of different chemicals used for IRS and ITNs, as well as the effects of combining the two methods, against important malaria vectors in south eastern Tanzania. I proposed also to develop a simple mathematical model to predict the community level outcomes of combining the methods in different situations; for example where there are different vector species, where different insecticides are used or where the vector populations are resistant to insecticides. This research therefore directly contributes towards the necessary evidence for day-to-day operations where ITNs and IRS are used either individually or in combination.

General objective of the PhD research

The overall objective of this study was to determine whether there is any added advantage in combining ITNs and IRS at household level and to recommend the most appropriate insecticides for combined use if there would be any scientific rationale for such combinations.

Specific objectives

1. To perform an in-depth review on: 1) the modes of action of insecticides used for IRS and ITNs and 2) potential benefits and limitations of combining LLINs and IRS in the same households (Chapter II)
2. To develop and optimize an experimental huts assay for evaluation of different LLINs and IRS insecticides and their combinations for malaria vector control (Chapter III).
3. To characterize and compare the different IRS insecticides and the different LLINs based on their modes of action against malaria vectors, and to compare effects of the individual interventions relative to various LLINs-IRS combinations, when used at household level (Chapters IV-V).
4. To develop and test a mathematical simulation that combines modes of action of different insecticides with behaviour of target malaria vectors to assess synergies and redundancies in community level effects of various LLIN-IRS combinations, applicable for malaria transmission control (Chapters VI-VIII).

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Chapter II

Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future *

Abstract

Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are currently the preferred methods of malaria vector control. In many cases, these methods are used together in the same households, especially to suppress transmission in holoendemic and hyperendemic scenarios. Though widespread, there has been limited evidence suggesting that such co-application confers greater protective benefits than either ITNs or IRS when used alone. Since both methods are insecticide-based and intradomiciliary, it is hypothesized that outcomes of their combination would depend on effects of the candidate active ingredients on mosquitoes that enter or those that attempt to enter houses. It is suggested here that enhanced household level protection can be achieved if the ITNs and IRS have divergent yet complementary properties, e.g. highly deterrent IRS compounds coupled with highly toxic ITNs. To ensure that the problem of insecticide resistance is avoided, the ITNs and IRS products should preferably be of different insecticide classes, e.g. pyrethroid-based nets combined with organophosphate or carbamate based IRS. The overall community benefits would however depend also on other factors such as proportion of people covered by the interventions and the behaviour of vector species. This article concludes by emphasizing the need for basic and operational research, including mathematical modelling to evaluate IRS/ITN combinations in comparison to IRS or ITNs alone.

* Adapted from: Okumu FO, Moore SJ: *Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future*. *Malaria Journal* 2011, 10(1):208

Background

Few vector control methods can be considered as effective against malaria mosquitoes as insecticide-treated nets (ITNs) and house spraying with residual insecticides (IRS). In recent years, endemic countries using the two methods singly or in combination have reported significant declines in malaria related morbidity and mortality [1-4]. A review of previous intervention trials has suggested that ITNs can reduce malaria cases by 39% to 62% and child mortality by 14% to 29% [5]. Similarly IRS has been shown to significantly disrupt malaria transmission, eliminate malaria vectors and reduce malaria incidence [1, 6-8]

Today, universal coverage with long lasting insecticide-treated nets (LLINs) or IRS is actively promoted as the main prevention strategy under the WHO endorsed malaria control and elimination plan [9, 10]. Where both ITNs and IRS are considered, the two methods are mostly used concurrently, within the same households, even though some national strategies emphasize one method more than the other [3]. Indeed, previous and current WHO guidelines have recommended the combination of ITNs and IRS in various malaria transmission scenarios, more so for holoendemic and epidemic situations [9, 11-13]. However, other than results from a small number of previous trials, which had varied primary objectives [14-16], there has not been any indisputable empirical evidence that ITN-IRS combinations can indeed offer any additional communal or personal protection, compared to using either method alone.

In this paper, recent trends of using ITNs and IRS are explored with special emphasis on: 1) significance of the two methods in current malaria control agenda, 2) potential benefits of combining the methods and 3) important research issues that

should be considered to support decision making regarding combination of these two methods.

Significance of IRS and ITNs in the current malaria control strategy

Other than intermittent preventive treatment (IPT), artemisinin-based combination therapy (ACT) and improved case detection by rapid malaria diagnostic tests (RDTs), recent declines of malaria are mostly attributable to expanded use of LLINs and IRS [2-4, 17, 18]. Today, these methods remain the mainstay of malaria control agenda, a situation which is likely to continue given the remarkably slow development and adoption of alternative interventions. Therefore, while the need for new vector control tools is being addressed, one of the greatest challenges is to optimize the ongoing use of existing ITNs and IRS through evidence-based decision making, and to ensure that any accrued successes are sustained.

The current Global Malaria Action Plan, recently launched by the WHO-Roll Back Malaria Partnership [9], targets universal coverage of all at-risk-populations with both preventive and curative measures. The idea is to scale up preventive measures to full coverage then sustain them at that point for extended periods, thus shifting malaria control dynamics towards elimination and possibly thereafter, complete eradication. This initiative is motivated mainly by evidence that malaria morbidity and mortality has been gradually, but steadily, reducing in many countries that have well organized control programmes [3, 11, 19]. Regarding vector control, this new action plan primarily advocates the use of long-lasting insecticidal nets (LLINs) and IRS, and to a small extent encourages use of other methods, depending on local evidence of effectiveness. To match these targets, production, distribution

and use of public health insecticides and LLINs are expected to grow exponentially. For example, it was originally approximated that 730 million LLINs would be distributed globally between 2008 and 2010, and that at least 350 million of these nets would go to Africa. In addition, 172 million households would be sprayed annually with insecticides [9].

On one hand, this new roadmap may be considered a realistic proposition given the proven effectiveness [1, 4-6, 20, 21] and the cost-effectiveness [22, 23] of the proposed methods, but also because of the gradually increasing government and donor funding for malaria control and research [3]. However, considering lessons learned from previous malaria campaigns, the targets may also be viewed as being overambitious and as exerting excessive pressure on poor malaria endemic countries, as well as on the donor community. So far even the WHO 2000 and 2005 malaria control targets [10, 24, 25] are yet to be met by many of these countries [3], and complete eradication is not deemed feasible in the short or medium term [26-28]. Moreover, the apparent over-reliance of the plan on insecticide-based methods is threatened by rise of insecticide resistance among target mosquito populations [29-32], which is known to have been one of the major reasons for the partial failure of malaria eradication programmes of the 1950s. Predictably, there is now a general consensus in the malaria control community that development of new vector control methods and new insecticides are key research priorities [33-37].

The WHO has provided guidelines for individual countries to use when prioritizing IRS, ITNs or both [38, 39]. For example in high transmission areas, it is recommended that children and pregnant women, who are most at risk, are preferentially covered while at the same time the countries should work towards ensuring that everyone gets and uses an insecticide-treated net. Moreover, in low

transmission areas, public health authorities should establish priorities based on the geographical distribution of malaria [38, 40]. One very significant shift from past practice is that long-lasting insecticide-treated nets (LLINs), which are designed to protect people for up to 3-5 years of use, are now being prioritized over ordinary ITNs, which have a far shorter duration of insecticidal activity [9, 38]. Indeed it is expected that only LLINs will be produced in future [9]. On the other hand, IRS, which was previously recommended for use in epidemic situations, in isolated communities and in low to moderate transmission areas, is now recommended also for high transmission areas [13, 39]. Perhaps most interesting, is the recognition that either ITNs or IRS if used alone may not be sufficient to disrupt malaria transmission, especially in holoendemic and hyperendemic areas, and that these two methods should preferably be combined in such situations [12, 38, 41].

Combining ITNs and IRS for malaria control

How widespread is combined use of ITNs and IRS in Africa?

Combining ITNs and IRS for malaria control has increasingly common in Africa. At the national level in sub-Saharan Africa, nearly all malaria endemic countries have adopted ITNs, IRS or both. Based on the latest world malaria report [3] more than twenty-five countries had policies involving both ITNs and IRS, including South Africa, which unlike most countries, preferentially promotes IRS over ITNs, the nets being saved for epidemic scenarios. About fifteen other countries were using ITNs but not IRS [3].

Typically, ITNs and IRS are not usually used in a mutually exclusive way. IRS is not always restricted to only households where ITNs are not already being

used, and the application of IRS itself does not always preclude use of ITNs. Instead, the two methods are commonly used together in the same communities or households. For example, a common application of IRS is in the mitigation of malaria epidemics [12, 13], where in many instances the residents already possess ITNs by the time IRS is launched.

Based on local evidence on malaria endemicity and other factors, such as financial costs and availability of storage and distribution systems, endemic countries often prioritize which regions should preferentially receive the different interventions. For example in Zambia, use of ITNs is targeted primarily in rural areas, while IRS is targeted primarily in urban and peri-urban areas [42], where spraying is likely to be more cost effective due to high densities of human populations. Zambia is also the only country that has ever expressly restricted mass distribution of ITNs to communities that are not eligible for IRS [43]. Nevertheless, even if promotion of IRS were restricted by government policy to areas where ITNs are not used, people may still obtain nets from the private sector or from non-governmental organizations.

What are the potential benefits of combining ITNs with IRS?

Despite the widespread implementation of ITNs and IRS and the likelihood of interactions between their properties, little is known about their impacts when they are used together. WHO has suggested that the two methods should be co-implemented to reduce transmission especially in hyperendemic and holoendemic scenarios [3, 38]. However, these recommendations are not entirely evidence-based as very little data are available from programs where both methods have been applied, or where combined ITN/IRS interventions have been evaluated relative to either method alone. Instead, most of the data available today come from large malaria control operations

conducted in communities where strategies included not only ITNs and IRS, but also other interventions including health education, artemisinin combination therapy, larviciding and environmental management [2, 15, 44]. Without direct measurements of transmission indicators (such as mosquito biting rates) and malaria burden indicators (such as incidence rates), from studies designed specifically to test the two vector control methods in combination, it is difficult to attribute observed protective benefits to any single intervention within the combined strategy as implemented in most of these previous large-scale interventions.

In Eritrea, where Nyarango *et al* evaluated the national malaria control programme between 2000 and 2004, there was no added advantage of using IRS and ITNs as opposed to using either method alone [44]. The authors argued that this might have been because the predominant vector in the region, *Anopheles arabiensis* was endophillic (indoor resting), and was, therefore, redundantly affected by ITNs and IRS since these interventions are both used indoors. In other words, the fact mere that both ITNs and IRS are indoor interventions, meant that there would be no additional benefit when they are applied against vector species are are also predominantly indoor feeding and indoor resting [44]. Elsewhere, in a retrospective evaluation of control operations between 1993 and 1999 in the Solomon Islands [15], where primary malaria vectors included *Anopheles punctulatus* and the exophilic (outdoor resting), early evening feeding *Anopheles farauti* [45], it was shown that reductions in malaria and fever incidences were associated not only with DDT house spraying, but also with ITNs and health education [15]. Though this particular appraisal did not directly measure combined effects of IRS and ITNs, it was established that ITNs could not possibly replace DDT-house spraying, but that the amount of the insecticide required would be reduced if ITNs were also used.

There are also reports showing that even though combinations of insecticidal nets with IRS lowered overall vector densities inside houses, there was no overall reduction in malaria transmission relative to situations where only one of the methods was used. Examples include reports by Protopopoff *et al* who evaluated the generally successful malaria control programme in the highlands of Burundi, where PermaNet 2.0™ nets, (deltamethrin treated LLINs), were deployed alongside very high coverage (90%) of deltamethrin and alpha-cypermethrin based IRS [46, 47]. In this project, the interventions were targeted both spatially and temporally, so as to focus on areas and times when transmission was highest [46-48].

More recently, Kleinschmidt *et al* completed a review of studies involving both IRS and ITNs [14]. Of the eight previous studies that they considered, five reported a reduced risk of infection in people protected by both interventions, compared to people protected with either IRS or nets alone. This research group also analysed results of household surveys conducted between 2006 and 2008 in Bioko, Equatorial Guinea and in Zambezi province, Mozambique [14], and found that in both places, the odds of contracting malaria were significantly lower for children living in houses with both IRS and ITNs, than for children living in houses with only IRS [14].

Mathematical modelling is also increasingly being adopted as a way of estimating potential benefits of combined ITN-IRS interventions, thereby partly filling the evidence gap while awaiting controlled field trials, but also enabling informed decision making by policy makers in areas where such co-applications are already being implemented [16, 41, 49]. In one case, based on simulations of IRS/ITN combined interventions, Yakob *et al* [16] recently reported that even though there is likely to be significant reduction of transmission by using 80% coverage with pyrethroid treated ITNs and DDT together at household level, this combination still

resulted in higher transmission potential (basic reproductive number, $R_0=11.1$ down from an control baseline of 39.5), than 80% coverage with just the ITNs alone without the DDT ($R_0=0.1$). Their explanations were that: 1) IRS compounds such as DDT, which have significant repellent properties reduce the likelihood that mosquitoes contact ITNs within the sprayed houses and 2) ITNs prevent mosquitoes from blood feeding and, therefore, reduce the rate at which blood fed mosquitoes rest on the walls [16]. This theoretical analysis seems to undermine the protective potential of the deterrent nature of IRS insecticides and somewhat contradicts actual field results from large scale vector control evaluations which have historically shown that high coverage with IRS using DDT results in significant reduction in community malaria risk [1, 6, 7].

Chitnis *et al* [49] also used a mathematical model to assess effectiveness of nets and IRS (with the organochloride, DDT or a carbamate, bendiocarb) when used singly or in combination, in a holoendemic area dominated by *Anopheles gambiae*. It should be noted that whereas DDT is proven to have significant repellency against mosquitoes [50-52], bendiocarb has minimal such effects [53]. Chitnis *et al* found that humans using only ITNs are generally better protected than those with only IRS, and that even though the ITNs or IRS with DDT provided similarly high personal protection, neither of them alone could interrupt transmission on its own [49]. Besides, they also showed that high coverage of IRS using bendiocarb alone might interrupt transmission as much as simultaneous high coverage of ITNs and IRS with DDT. This finding indicates that the key question is not only whether people use IRS, ITNs or both, but that it is also imperative to consider the type of insecticides (i.e. active ingredients) used in these interventions. One other crucial suggestion from this research group was that IRS and net combinations would be most effective if the

second intervention being introduced is initially targeted at those people who are not yet covered by the existing intervention [49].

Other than actual efficacy of individual insecticides, there are several other factors associated with the overall performance of these intradomiciliary interventions and their combinations. For example, a comprehensive model-based evaluation of interventions showed that in low endemicity areas, where people experience approximately three infectious mosquito bites per year (annual EIR~3) or less, LLINs alone can drive malaria transmission to levels below the 1% parasite prevalence threshold necessary to start pursuing elimination [41]. However, the same model also predicted that, in moderate transmission areas (annual EIR between 43 and 81), additional interventions such as IRS with DDT and mass screening and treatment of malaria cases, would be required alongside LLINs to achieve the same target [41]. The situation gets more complicated when the malaria vector is more exophilic (outdoor resting) than endophilic (indoor resting). It has been suggested that in these areas and also in areas with high transmission (EIR in the range of hundreds or more), existing interventions, even if combined, cannot completely disrupt malaria transmission [41]. As such additional interventions especially those that target outdoor-feeding or outdoor-resting mosquitoes will be required to achieve these targets [35, 37, 41].

Where ITN and IRS insecticides have overlapping modes of action, insecticide combinations may remain protective for much longer than when only a single insecticide is used. Such an observation is exemplified in the work reported by Protopopoff *et al* in Burundi, where LLINs were provided to continue protecting people even after the residual activity of the IRS insecticides had ceased to be effective [46, 47]. This concept of extending insecticide persistence can also be

explained by results from studies where two different IRS insecticides were applied in same houses. In one study, Service *et al* reported that huts sprayed with both Malathion and DDT remained toxic to mosquitoes much longer and that these huts were less irritant against both *Anopheles funestus* and *An. gambiae* than huts sprayed with just DDT [52]. There are also reports from the IRS program in New Guinea in the 1950s, where pure DDT was replaced by a mixture of DDT and dieldrin in selected areas with persistently high transmission [54]. Though additional transmission reduction was observed, it could not be confirmed to be a direct result of the change of interventions. The original idea however was that the long residual effect of the DDT together with the high initial toxicity of dieldrin would be able to achieve better control of malaria than just pure DDT [54, 55]. Even though existing IRS compounds last for only a few months, with the exception of DDT that lasts 6-12 months on sprayed walls [56], sustainable ITN/IRS strategies will require advanced technologies to develop long lasting formulations for IRS such as those recently tested in west Africa [36], which could achieve even greater benefits when combined with LLINs.

Based on reports analysed above, it seems that at least in some cases, there are advantages of combining ITNs with IRS relative to using either method alone, but that this outcome may be different in certain situations, since there are numerous confounding factors that can affect the results. It is therefore certain that evidence to support or refute this strategy of combinations remains inconclusive and any generalizations for optimal strategies cannot be made.

A functional description of insecticides commonly used for IRS and ITNs, and its relevance in selecting candidate insecticides for use in combined ITN/IRS interventions

In practice, the decision to use IRS, ITNs or both methods should be based on existing epidemiological conditions, operational requirements and expected protective efficacy of the interventions. The protective efficacy is itself a function of several other factors including behaviour of the local mosquito populations and presence or absence of insecticide resistance among these vectors. Both IRS and ITNs are insecticide-based and they both target mosquitoes that enter or those that attempt to enter human dwellings (Figure 1). The WHO has approved 12 different insecticides for IRS and six for use on bed nets [56]. Two of these insecticides, deltamethrin and alpha cypermethrin can be used for both bed nets and IRS [56].

Each insecticide elicits a distinct spectrum of behavioural and physiological outcomes on mosquitoes, implying that ITNs and IRS, if based on different insecticides could differentially affect vectors even if they are simultaneously used in the same house. In this section, data from previous studies on house spraying and insecticide treated nets are considered to enable a generalised description of these interventions on the basis of how each one of them can affect mosquitoes that enter or those that attempt to enter human occupied houses (Tables 1-3). This functional description is then used to briefly illustrate how best one could select appropriate insecticides for a combined ITN-IRS intervention. The studies considered here were all conducted in areas with susceptible populations of anthropophilic malaria vectors *An. gambiae* and *An. funestus*, in special experimental huts designed to mimic local human houses [57].

Despite some differences in terminology [58-60], insecticides can be described generally as: 1) *deterrents or spatial repellents*, if they prevent mosquitoes from entering houses [59, 61-63], 2) contact *irritants*, if they force mosquitoes that contact treated surfaces in the houses to exit, usually earlier than they normally would [59, 61, 64] or 3) *toxicants*, if they kill mosquitoes that contact treated surfaces or insecticide fumes [59]. In addition, insecticides may inhibit the ability of mosquitoes to take blood meals, i.e. *feeding inhibition* [65], or reduce chances of a mosquito surviving after non-lethal contacts, i.e. *sub-lethal effects* [63, 66].

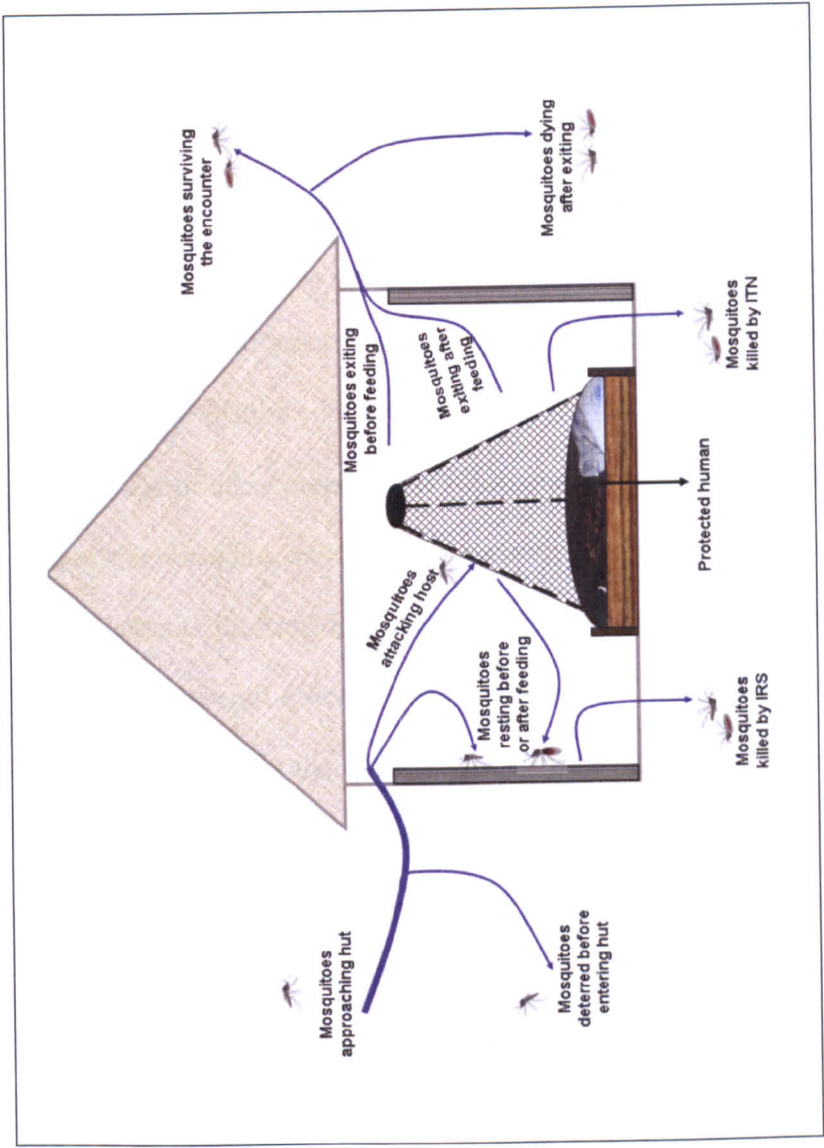


Figure 1: a diagrammatic representation of various effects of ITNs and IRS on mosquitoes that enter or attempt to enter houses. Insecticides used on nets or for IRS effect mosquitoes at different levels along the path towards the individual human inside the sprayed hut. Mosquitoes can be deterred and diverted before they enter houses, killed by the IRS or ITNs, or they can be irritated so that they exit the huts earlier than normal. Exit may occur before or after the mosquitoes have fed, but both the fed and the unfed mosquitoes may die later after they have left the huts due to sub-lethal effects of the ITN or IRS insecticides. The net and the IRS may also inhibit mosquitoes' ability to successfully take blood meals from the hut dwellers.

Computationally, deterrence or spatial repellence is calculated as the difference between number of mosquitoes entering treated huts and number entering control huts presented as a percentage of the number entering the control hut. Feeding inhibition is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed and toxicity, as the percentage of mosquitoes entering the treated hut that die. Because in most previous studies, mosquitoes were sampled once a night as opposed to several times a night e.g. hourly, it is not possible to accurately derive values for contact irritancy based on the definition used in this article. The term excess exit is, therefore, used as a simplification for contact irritancy [59], and is calculated as the difference between percentage of mosquitoes exiting the treated huts and percentage exiting control huts.

Each of these properties is functionally applicable at different levels along the path of the mosquito, as it approaches a net-user inside an insecticide sprayed house. This process is illustrated in detail in Figure 1. Nevertheless, the properties together contribute to overall efficacy of the insecticide-based interventions. It can be argued that any interventions that reduce man vector contact and vector survival, whether by killing or by deterring host-seeking mosquitoes from potential blood sources, will subsequently also reduce the probability of mosquito-borne disease transmission [67]. Therefore even though direct toxicity has been the most desired property of public health chemicals [1], combined IRS/ITN interventions could confer superior protection against malaria at household level if the constituent applications have additional properties such as deterrence. In one example where Cullen and de Zulueta [50] were reporting on effects of DDT on malaria vectors in Uganda, they explained that the fate of mosquitoes deterred from experimental huts is intriguing in the sense that they may find food or shelter elsewhere, but also that they may die from a

combination of factors such as starvation, predation and exposure to harsh environmental conditions [50]. Nevertheless, these scientists went ahead to affirm that the crucial contact between mosquitoes and humans, which is required for malaria transmission to take place between humans and mosquitoes, is reduced even without any direct toxicity [50].

Based on results outlined in Tables 1-3, it can be argued that while the efficacy of IRS applications is mainly due to repellency and toxicity to mosquitoes, ITNs (including LLINs) mainly inhibit feeding and kill mosquitoes. In selective cases such as when the nets are treated with permethrin, their effects can include moderate levels of repellency to the mosquitoes. It appears also that effects of insecticidal applications are augmented, moderately by their ability to inhibit blood feeding by the vectors and also the fact that they can irritate and force mosquitoes to leave houses in excess numbers. From many previous experimental hut studies, IRS with DDT or lambda cyhalothrin consistently conferred >50% deterrence (Table 1). However, bendiocarb, a carbamate commonly used for IRS, appears to be highly toxic to susceptible mosquitoes and to have significant feeding inhibition, yet it confers only limited deterrence [53, 68]. This particular compound is often proposed as a potential alternative for use against insecticide resistant populations [53, 68].

Insecticidal nets are effective mainly because they prevent blood feeding, even when nets become torn and also because they kill the vectors. Unlike in the case of IRS, deterrence is not a major property of LLINs (Table 2). Most of the previous studies suggest that LLINs in particular elicit either very low levels of deterrence or no deterrence at all against susceptible African malaria vectors [69-74]. However, home-treated nets (also commonly referred to as conventionally treated nets) appear to consistently confer moderate levels of insecticide associated deterrence [69, 72-78],

even though there is one study with evidence to show that such effects may actually be due to the insecticide carrier medium and not the insecticide *per se* [77]. It is likely that IRS conveys higher deterrence than ITNs because IRS applications utilize higher quantities of insecticides, resulting in higher concentrations of the insecticide in IRS-huts than in huts containing bed nets treated with the same insecticides. This situation notwithstanding, many of these previous studies also show that IRS confers only moderate feeding inhibition (Table 1), and as such the intervention alone may not be adequate to prevent transmission within households. Thus, additional interventions such as nets should be incorporated to enhance personal protection at household level.

Another concern regarding IRS is the rapid decay of the associated insecticidal efficacy with time. For example, while DDT-sprayed houses would not need to be re-sprayed until after 6 to 12 months, houses sprayed with pyrethroids, such as lambda cyhalothrin, must be retreated every 3-4 months to maintain acceptable efficacies [56]. Again, since this retreatment may not always be feasible, addition of LLINs is highly desirable and should be considered in such households with IRS, so that the people can continue to be protected even after the IRS insecticide has been depleted. Indeed new generation LLINs are made to last between 3-5 years and studies have now demonstrated continued efficacy of these nets after several years of use [73, 74, 79].

Table 1^N. Effects of insecticides commonly used for indoor residual spraying (IRS) in Africa on mosquitoes that enter or those that attempt to enter human occupied huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit [†]

| Insecticide | Country | Major vector | Dosage | Duration | Deterrence (%) | Feeding inhibition (%) | Toxicity (%) | Excess % exit | Reference |
|-----------------------|--------------------------|---|-------------------------|----------|----------------|------------------------|--------------|---------------|--------------------|
| DDT | Uganda | <i>An. funestus</i> | 2g/ m ² WP | 7 months | 80.2 | - | 71.0 | - | [50] |
| | | | 2g/ m ² WP | 7 months | 88.9 | - | 83.6 | - | |
| | | | 2g/ m ² WP | 7 months | 81.4 | - | 50.9 | - | |
| | | | 2g/ m ² WP | 7 months | 95.2 | - | 79.8 | - | |
| | | | 2g/ m ² WP | 7 months | 74.4 | - | - | - | [50] |
| | Nigeria South Africa | <i>An. gambiae</i> | 2g/ m ² WP | 7 months | 89.0 | - | - | - | |
| | | | 2g/ m ² WP | 9 months | 69.5 | - | - | - | [85] |
| | | | 2g/ m ² WP | 6 months | 68.1 | - | 52.0 | 50.3 | [52] [^] |
| | | | 2g/ m ² WP | 3 months | 52.4 | 67.1 | 55.9 | 26.3 | [51] [^] |
| | | | 2g/ m ² WP | 3 months | 96.6 | 0.0 | - | - | [51] ^{^*} |
| Lambda cyhalothrin | Tanzania Kenya | <i>An. gambiae</i> <i>An. gambiae</i> <i>An. funestus</i> | 2g/ m ² WP | 5 months | - | 36.0 | 32.0 | - | [88] [^] |
| | | | 2g/ m ² WP | 5 months | 56.4 | 35.1 | 17.0 | 46.2 | [86] ⁺ |
| | | | 2g/ m ² WP | 6 months | 50.3 | 13.9 | 15.7 | 32.3 | |
| | Benin | <i>An. gambiae s.l</i> <i>An. gambiae</i> | 2g/ m ² WP | 6 months | 36.3 | 22.1 | 41.2 | 8.75 | |
| | | | 0.03g/m ² CS | 3 months | 20.7 | 25.8 | 72.1 | 8.9 | [30] |
| Bendiocarb | South Africa Tanzania | <i>An. arabiensis</i> <i>An. gambiae & An. funestus</i> | 0.03g/m ² CS | 6 months | 50.0 | 8.8 | 8.8 | - | [71] |
| | | | 0.03g/m ² CS | 5 months | 56.3 | 39.0 | 48.0 | - | [88] [^] |
| | | | 0.03g/m ² CS | 7 months | 71.7 | 59.0 | - | - | [87] |
| | Benin | <i>An. gambiae s.s</i> | 0.02g/ m ² | 2 Months | 20.8 | 87.5 | 92.9 | 10.0 | [53] |

^N Additional notes relevant to this are found on the next page.

Additional notes on Table 1

¶ This table includes a section of studies conducted in Africa, in areas where no resistance against DDT or pyrethroids had been reported. In studies where parameter values were not explicitly stated in the original publication, these values have been calculated from summary tables given in those original publications. **Deterrence** is calculated as the difference between number of mosquitoes entering treated huts and number entering control huts and is presented as a percentage of the number entering the control hut. **Feeding inhibition** is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed and **toxicity**, as the percentage of mosquitoes entering the treated hut that die. **Excess exit** is derived as the difference between percentage exit rates in sprayed and unsprayed huts, based on values presented in the original publications. The column for **duration** refers to the period after spraying, for which the data included in the analysis was collected.

^ Studies by Service *et al* 1964 [52] and Sharp *et al* 1990 [51] were conducted in local houses fitted with exit traps, unlike in all the other studies where specially designed experimental huts were used.

• * Only mosquitoes collected from the floors are included in this row

+ The formula used by Smith and Webley [86] , to calculate deterrence is slightly different from that used in the other publications. That is, instead of using parallel catches in control huts as the reference, deterrence is determined by comparing number of mosquitoes entering treated huts with an expected number (N), which is calculated as $N = (C \times E)/C_1$, where C is the number of mosquitoes entering control hut after spraying, E is the number entering treated hut after spraying and C_1 is the number entering control hut prior to the spraying of any hut. Also the results presented here are averages for all the months during which the experiments were conducted and may not exactly match the summary values in the original publication. For example, it should be noted that the deterrence value stated in the original publication is 60-70% which excludes the first month of the study.

♣ The study on the carbamate, Bendiocarb, was conducted in an area with high frequency of pyrethroid resistance, but with no resistance against the carbamates themselves [53], thus permitting its inclusion in this review, which otherwise considered only studies in areas where mosquitoes were susceptible to DDT and pyrethroids.

Table 2a^N. Effects of conventionally treated nets (ordinary home-treated ITNs) commonly used in Africa on mosquitoes that enter or those that attempt to enter human huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit[‡]. The nets are grouped as per the active ingredients (insecticides) used to treat them.

| Insecticide | Country | Major Vector | Washing | Dosage | Holes | Deterrence (%) | Feeding inhibition (%) | Toxicity (%) | Excess % exit | Reference |
|--------------------|------------|---|----------|----------------------|-------|----------------|------------------------|--------------|---------------|-------------------|
| Alpha cypermethrin | The Gambia | <i>An. gambiae</i> s.l | Unwashed | 100mg/m ² | Yes | 0 | 92.0 | 94.0 | - | [78] |
| | | | Washed | 100mg/m ² | Yes | 0 | 91.0 | 74.0 | - | |
| | Tanzania | <i>An. arabiensis</i> | Unwashed | 25mg/m ² | Yes | 25.0 | 82.6 | 32.8 | 1.9 | [72] [‡] |
| | | | Unwashed | 10mg/m ² | Yes | 45.8 | 81.5 | 59.5 | - | [96] |
| | | <i>An. gambiae</i> & <i>An funestus</i> | Washed | 10mg/m ² | Yes | 27.9 | 67.7 | 24.8 | - | |
| | | | Unwashed | 20mg/m ² | Yes | 21.2 | 68.5 | 63.4 | - | |
| | | | Washed | 20mg/m ² | Yes | 13.6 | 66.7 | 43.5 | - | |
| | | | Unwashed | 40mg/m ² | Yes | 11.4 | 79.1 | 50.1 | - | |
| | | | Washed | 40mg/m ² | Yes | 44.2 | 79.2 | 43.5 | - | |
| | | | Unwashed | 20mg/m ² | Yes | 21.1 | 67.9 | 72.0 | 3.5 | [74] |
| | | | Unwashed | 20mg/m ² | Yes | 32.7 | 69.3 | 70.6 | 8.4 | |
| | | <i>An. gambiae</i> | Washed | 20mg/m ² | Yes | 0 | 29.9 | 69.6 | 6.0 | |
| | | | Washed | 20mg/m ² | Yes | 7.7 | 9.9 | 58.4 | 4.8 | |

^N Additional notes relevant to this are found on the page after Table 2c.

Table 2b^N. Continued from Table 2a: Effects of conventionally treated nets (ordinary home-treated ITNs) commonly used in Africa on mosquitoes that enter or those that attempt to enter human huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit[†]. The nets are grouped as per the active ingredients (insecticides) used to treat them.

| Insecticide | Country | Major Vector | Washing | Dosage | Holes | Deterrence (%) | Feeding inhibition (%) | Toxicity (%) | Excess % exit | Reference |
|-------------|------------|--|----------|-----------------------|-------|----------------|------------------------|--------------|---------------|-------------------|
| Permethrin | Tanzania | <i>An. arabiensis</i> | Unwashed | 200mg/m ² | Yes | 33.7 | 72.0 | 49.8 | - | [75] ⁺ |
| | | | Unwashed | 200mg/m ² | No | 20.6 | 61.0 | 41.9 | - | |
| | | | Unwashed | 80mg/m ² | No | 10.6 | 71.2 | - | 28.3 | [76] ^c |
| | | <i>An. arabiensis</i> | Unwashed | 25mg/m ² | Yes | 35.3 | 85.8 | 15.2 | 5.9 | [72] |
| | | | Unwashed | 200mg/m ² | No | 57.1 | 75.0 | 89.0 | 27.0 | [76] |
| | | | Unwashed | 1000mg/m ² | No | 66.6 | 63.0 | 70.0 | 56.0 | |
| | | <i>An. gambiae</i> & <i>An. funestus</i> | Unwashed | 200mg/m ² | Yes | 38.7 | 97.8 | 46.3 | - | [73] |
| | | | Unwashed | 200mg/m ² | Yes | 20.5 | 82.2 | 29.8 | - | |
| | Kenya | <i>An. gambiae</i> | Unwashed | 500mg/m ² | No | 15.0 | 83.9 | - | 50.8 | [97] ^γ |
| | | <i>An. arabiensis</i> | Unwashed | 500mg/m ² | No | 0 | 66.7 | - | 13.9 | |
| | | <i>An. funestus</i> | Unwashed | 500mg/m ² | No | 35.7 | 85.9 | - | 49.6 | |
| | | <i>An. gambiae</i> s.s | Unwashed | 500mg/m ² | No | 94.6 | - | - | - | [98] ^γ |
| | | <i>An. funestus</i> . | Unwashed | 500mg/m ² | No | 96.7 | - | - | - | |
| | The Gambia | <i>An. gambiae</i> s.l. | Unwashed | 5mg/m ² | Yes | 33.0 | 96.3 | 74.0 | 2.0 | [77] ^λ |
| | | <i>An. gambiae</i> s.l. | Unwashed | 50mg/m ² | Yes | 45.1 | 98.2 | 75.0 | 4.0 | |
| | | <i>An. gambiae</i> s.l. | Unwashed | 500mg/m ² | Yes | 69.9 | 98.7 | 79.0 | 10.0 | |

^N Additional notes relevant to this are found on the page after Table 2c.

Table 2c^N. Continued from Table 2b. Effects of conventionally treated nets (ordinary home-treated ITNs) commonly used in Africa on mosquitoes that enter or those that attempt to enter human huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit^ε. The nets are grouped as per the active ingredients (insecticides) used to treat them.

| Insecticide | Country | Major Vector | Washing | Dosage | Holes | Deterrence (%) | Feeding inhibition (%) | Toxicity (%) | Excess % exit | Reference |
|--------------|------------|---|----------|----------------------|-------|----------------|------------------------|--------------|---------------|-------------------|
| Lambda | The Gambia | <i>An. gambiae</i> s.l. | Unwashed | 25mg/m ² | Yes | 33.3 | 97.8 | 89.0 | 0 | [77] ² |
| Cyhalothrin | Tanzania | <i>An. gambiae</i> & <i>An. funestus</i> | Unwashed | 10mg/m ² | Yes | 33.6 | 63.3 | 71.4 | - | [96] |
| | | | Washed | 10mg/m ² | Yes | 31.8 | 54.8 | 61.3 | - | |
| | | | Unwashed | 20mg/m ² | Yes | 32.6 | 63.3 | 74.8 | - | |
| | | | Washed | 20mg/m ² | Yes | 23.0 | 62.3 | 56.0 | - | |
| | | | Unwashed | 18mg/m ² | Yes | 26.4 | 96.1 | 98.5 | 10.7 | [30] |
| Deltamethrin | The Gambia | <i>An. gambiae</i> s.l. | Unwashed | 25mg/m ² | Yes | 11 | 93 | 88 | - | [78] |
| | | | Washed | 25mg/m ² | Yes | - | 87 | 74 | - | |
| | | | Unwashed | 500mg/m ² | Yes | 60 | 98 | 72 | - | |
| | | | Washed | 500mg/m ² | Yes | - | 87 | 54 | - | |
| | | | Unwashed | 25mg/m ² | Yes | 22 | 98 | 86 | - | |
| | | | Washed | 25mg/m ² | Yes | 0 | 87 | 87 | - | |
| | | | Unwashed | 25mg/m ² | Yes | 30.7 | 81.4 | 33.0 | 2.5 | [72] |
| | | | Washed | 25mg/m ² | Yes | 22.5 | 89.0 | 69.0 | 6 | [69] |
| | Tanzania | <i>An. arabiensis</i> <i>An. gambiae</i> | Unwashed | 25mg/m ² | No | 0 | 90.3 | 83.9 | - | [70] |
| | | | Washed | 25mg/m ² | No | 0 | 91.2 | 70.2 | - | |
| | | | Washed | 25mg/m ² | No | 0 | 95.2 | 88.0 | - | |
| | | | | | | | | | | |
| | | | | | | | | | | |

^N Additional notes relevant to this are found on the next page.

Additional notes relevant to Tables 2a-c

[‡] This table includes a section of studies conducted in Africa, in areas where no resistance against DDT or pyrethroids had been reported. In studies where parameter values were not explicitly stated in the original publication, these values have been calculated from summary tables given in those original publications. **Deterrence** is calculated as the difference between number of mosquitoes entering treated huts and number entering control huts and is presented as a percentage of the number entering the control hut. **Feeding inhibition** is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed. For purposes of uniformity, this formula was also applied to recalculate feeding inhibition for those studies where the authors had originally corrected the percentage feeding rates in treatment huts on the basis of feeding rates in control huts e.g. in Tungu *et al.*, 2010 [69]. **Toxicity** on the other hand has been calculated as the percentage of mosquitoes entering the treated hut that die and **excess exit** is derived as the difference between percentage exit rates in sprayed and unsprayed huts, based on values presented in the original publications.

[§] In the study by Mosha *et al* 2008 [72], the percentage mortality observed among mosquitoes collected in control huts was greater than 20%, therefore the toxicity values represented here are statistically corrected percentages.

⁺ In studies by Lines *et al* 1985 and Lines *et al* 1987, the vector species are reported as *An. gambiae s.l.* though the original publications also had statements indicating that these mosquito populations were almost entirely *An. arabiensis* [75, 76].

^c Results represented in this raw from the study by Lines *et al* [76] were obtained from tests of nets made of cotton rather than polyester as used in the rest of the studies

^λ Deterrence and feeding rates in the Lindsay *et al.*, 1991 paper were recalculated, by subjecting the log numbers presented in the original publication to a microsoft excel function ($z = \text{IMEXP}$) that returns the actual number of mosquitoes (z) as an exponential of complex numbers originally in $x + y_i$ or $x + y_j$ format.

[‡] In the studies by Mathenge *et al.*, 2001[97] and Bogh *et al.*, 1998[98], the data used was based on pyrethrum spray catches done inside local huts and also from catches of exiting mosquitoes trapped using Colombian curtains [57] installed around village huts that were allocated (or not allocated) nets.

Table 3a^N: Effects of different long lasting insecticidal nets (LLINs) commonly used in Africa, on mosquitoes that enter or those that attempt to enter human occupied huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit^ε.

| Type [‡] | Insecticide | Country | Major Vector | Washing | Holes | Deterrence (%) | Feeding inhibition (%) | Toxicity (%) | Excess % exit | Reference |
|-------------------|--------------------|----------|--|----------|-------|----------------|------------------------|--------------|---------------|-------------------|
| PermaNet 2.0™ | Deltamethrin | Tanzania | <i>An. gambiae</i> | Unwashed | Yes | 20.6 | 90.0 | 95.0 | 0 | [69] |
| | | | | Washed | Yes | 18.9 | 91.0 | 85.0 | 2 | |
| | | | <i>An. gambiae</i> | Unwashed | No | 0 | 93.0 | 97.7 | - | [70] |
| | | | | Washed | No | 0 | 96.4 | 96.6 | - | |
| | | | <i>An. gambiae</i> & <i>An. funestus</i> | Unwashed | No | 0 | 93.4 | 85.5 | - | [70] |
| | | | | Washed | No | 0 | 98.2 | 93.0 | - | |
| PermaNet 3.0™ | Deltamethrin | Tanzania | <i>An. gambiae</i> | Unwashed | Yes | 41.2 | 97.0 | 95.0 | 0 | [69] |
| | | | | Washed | Yes | 22.8 | 90.0 | 94.0 | 0 | |
| Interceptor™ | Alpha cypermethrin | Benin | <i>An. gambiae</i> s.l | Unwashed | No | 22.5 | 90.0 | 95.0 | 22.5 | [71] ⁸ |
| | | | | Washed | No | 22.5 | 90.0 | 95.0 | 22.5 | |
| | | Tanzania | <i>An. gambiae</i> | Unwashed | No | 0 | 88.0 | 93.0 | 15.0 | |
| | | | | Washed | No | 0 | 82.0 | 73.0 | 15.0 | |
| | | | <i>An. funestus</i> | Unwashed | No | 0 | - | 76.0 | - | |
| | | | | Washed | No | 0 | 86.0 | 60.0 | - | |
| | | | <i>An. gambiae</i> s.l. | Unwashed | No | - | 93.0 | 88.0 | - | |
| | | | | Washed | No | - | 79.0 | 84.0 | - | |
| | | | <i>An. funestus</i> | Unwashed | No | - | 67.0 | - | - | |
| | | | | Washed | No | - | 61.0 | 96.0 | - | |

^N Additional notes relevant to this are found at the end of Table 3b.

Table 3b ^N **Continued from Table 3a:** Effects of different long lasting insecticidal nets (LLINs) commonly used in Africa, on mosquitoes that enter or those that attempt to enter human occupied huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit [‡].

| <i>Type</i> [‡] | <i>Insecticide</i> | <i>Country</i> | <i>Major Vector</i> | <i>Washing</i> | <i>Holes</i> | <i>Deterrence</i> (%) | <i>Feeding inhibition</i> (%) | <i>Toxicity</i> (%) | <i>Excess exit</i> % | <i>Reference</i> |
|--------------------------|--------------------|----------------|---|----------------|--------------|--------------------------|----------------------------------|------------------------|-------------------------|-------------------|
| Olyset™ | Permethrin | Tanzania | <i>An. arabiensis</i> | Unwashed | Yes | 0 | 96.3 | 11.8 | 25.6 | [72] [‡] |
| | | | <i>An. gambiae</i> & <i>An. funestus</i> | Unwashed | No | 5.4 | 87.2 | 56.0 | - | [73] |
| | | | | Unwashed | No | 0 | 90.3 | 55.0 | - | |
| | | | | Washed | No | 0 | 97.2 | 70.0 | - | |
| | | | | Unwashed | No | 0 | 80.4 | 49.0 | - | |
| | | | <i>An. gambiae</i> | Unwashed | Yes | 0 | 40.9 | 62.7 | 7.2 | [74] |
| | | | <i>An. funestus</i> | Unwashed | Yes | 28.9 | 49.9 | 73.9 | 1.4 | |
| | | | <i>An. gambiae</i> & <i>An. funestus</i> | Washed | No | 0 | 81.1 | 57.5 | - | [73] [‡] |
| | | | | Washed | Yes | 0 | 0 | 40.0 | 5.9 | [74] [‡] |
| | | | | Washed | Yes | 30.8 | 0 | 58.9 | 4.2 | |

^N Additional notes available below

Additional notes relevant to tables 3a and 3b

[‡] This table includes a section of studies conducted in Africa, in areas where no resistance against DDT or pyrethroids had been reported. In studies where parameter values were not explicitly stated in the original publication, these values have been calculated from summary tables given in those original publications. **Deterrence** is calculated as the difference between number of mosquitoes entering treated huts and number entering control huts and is presented as a percentage of the number entering the control hut. **Feeding inhibition** is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed. For purposes of uniformity, this formula was also applied to recalculate feeding inhibition for those studies where the authors had originally corrected the percentage feeding rates in treatment huts on the basis of feeding rates in control huts e.g. in Tungu *et al.*, 2010 [69]. **Toxicity** on the other hand has been calculated as the percentage of mosquitoes entering the treated hut that die and **excess exit** is derived as the difference between percentage exit rates in sprayed and unsprayed huts, based on values presented in the original publications.

‡ **PermaNet 2.0™** is a 00% polyester LLIN coated with 55-62mg of synthetic deltamethrin per square metre. **PermaNet 3.0™** on the other hand is a mosaic-style LLIN specifically designed for the control of insecticide resistant mosquito populations. Its side panels, which unlike PermaNet 2.0™ have strengthened borders, are made of deltamethrin-coated-polyester (with approximately 118 mg/m² of deltamethrin), while the top panel is made of monofilament polyethylene fabric into which a higher dose of deltamethrin (approx. 180 mg/m²) and approximately 1100mg/m² of a synergist, piperonyl butoxide (PBO) are incorporated. This synergist inhibits mixed function oxidases, which are known to be associated with pyrethroid resistance. PermaNet 3.0™ is also manufactured by Vestergaard Frandsen, Denmark. **Interceptor™** is a long lasting insecticidal net made of polyester coated with alpha cypermethrin (200mg/m²). It is manufactured by BASF, Germany. Finally, **Olyset™** is made of a polyethylene netting (150 deniers), that is impregnated during manufacture with synthetic permethrin at a concentration of 2% (equivalent to 1000mg of active ingredient per square metre). It is manufactured by A to Z company, Tanzania.

‡ The results for Interceptor™ nets evaluation in Benin are reported in the WHO report in very general terms as follows: high mortality (above 95%), high blood feeding inhibition (above 90%), 15-30% deterrence and 10-35% increase in exophilly [71]. Values reported in this table are therefore estimated as minimum mortality (95%) minimum feeding inhibition (90%), mean deterrence (22.5%) and mean excess exit (22.5%).

§ In the study by Mosha *et al* 2008 [72], the percentage mortality observed among mosquitoes collected in control huts was greater than 20%, therefore the toxicity values represented here are statistically corrected percentages.

¶ The data represented in these specific rows were collected from studies where the Olyset™ nets tested had already been in use for 4 years [73] or 7 years [74].

Another important element in the studies considered in Tables 2-3 is the effect of wear and tear and also the effect of washing on insecticidal nets. Contrary to what may be expected, it is not clear from existing research evidence (Tables 2-3) that feeding inhibition is reduced when insecticidal nets are torn. It should be noted however that in most of these studies, it was not originally intended to compare torn versus intact nets, but rather the investigators used either only torn nets or only intact nets. On the other hand, while washing of nets seem to consistently reduce toxicity of conventionally treated nets, this is not the case with LLINs (Table 3). Indeed there is at least one study with limited evidence to suggest that washed Olyset™ nets killed slightly more *An. gambiae* mosquitoes than unwashed nets [73] perhaps because the process of washing releases insecticide from within the net fibres to the surface where the insecticide may contact resting mosquitoes.

Lastly, variations in efficacy of IRS or nets are seemingly dependent on modes of action of actual active ingredients used. For example, considering IRS, it is clear from studies listed in Table 1 that DDT has higher deterrence than both lambda cyhalothrin and bendiocarb. It can also be said that of all insecticides used in home-treated nets, permethrin appears to be the least toxic yet the most deterrent and also most irritating to mosquitoes (Table 2). Moreover, results from some early research in the Gambia indicates that the deterrence property of ITNs was mainly a result of the emulsifiable concentrates used for hand treating these nets [77], an argument which could also explain why such deterrence is limited in the case of LLINs, where the insecticide is actually impregnated into the net fibers or coated with resins onto the nets. Such differences are however not very obvious between LLINs, except that Olyset nets tend to kill fewer vectors than the other LLINs (Table 3).

An important inference from this review is that toxicity to mosquitoes is not always the most significant attribute of insecticidal nets or IRS applications. There are many instances where protection is mainly due to other properties such as deterrence and feeding inhibition as opposed to simply the killing of the mosquitoes. Whereas toxic insecticidal applications arguably remain preferable in achieving mass community effects by reducing populations of biting mosquitoes [1, 80-82], high coverage with repellent applications such as DDT would achieve similar community level effects by starving mosquitoes of human sources of blood, thus increasing foraging related mortality, and reducing lifetime mosquito fecundity especially in communities where there are no alternative blood hosts [6, 7, 83]. Thus these results also have crucial implications regarding intervention coverage and delivery systems.

This functional description can be used to improve decision-making regarding which insecticides to use when combining ITNs and IRS. Based on data from previous IRS and net applications (Tables 1-3), there are at least two reasons to combine the interventions. The first reason is to expand coverage and or prolong the protection even after one of the interventions is weakened, for example LLINs can be used to ensure protection long after IRS insecticides have decayed [46, 47]. Similarly IRS can enhance protection in households where the nets being used are worn old, torn and have been repeatedly washed (Table 2), or where some individual members of the house hold do not use the nets [84]. The second reason is to provide additional level of protection at the household level (Figure 1), for example IRS compounds with significant deterrence e.g. DDT [50, 85, 86] or lambda cyhalothrin [87, 88] can provide an additional level of protection in households where there is a purely toxic net, or a toxic net with minimal deterrent effects e.g. PermaNet 2.0™ [69, 70]. That way, effects of the combined intervention are boosted at all the stages as the mosquito

approaches the net user inside sprayed house (Figure 1). Such a combination would have high deterrence (from the IRS), high mortality (from both the IRS and the ITNs) and high feeding inhibition (from the ITNs), thus significantly improving the overall effects upon vectors. If sufficiently high coverage is achieved, benefits accrued from such enhanced household level protection should lead to improved community level protection as well. Notwithstanding the argument that high deterrence could simultaneously reduce probability of mosquitoes contacting insecticides thus lowering household mortality rates and overall community benefits [16], it should be noted that in situations where mosquito vectors are highly anthropophilic e.g. *An. funestus* and *An. gambiae sensu stricto*, consistently diverting them from human dwellings, for example by spraying DDT in most dwellings in an area, has been shown to dramatically reduce vector populations and malaria transmission, as these anthropophilic vectors have few other blood sources to rely upon [1, 6, 7, 85].

Important research questions concerning combination of ITNs and IRS

The sections above have highlighted the fact that whereas IRS and ITNs continue to be used both singly and in combination, the current state of affairs is that it is still an open question as to whether there is any added advantage of combining the interventions. Review of previous studies has also shown that given the differences in modes of action of various IRS compounds and net types, it is likely that certain combinations may be carefully selected that result in an improved overall protection that use of either nets alone or IRS alone. But no such combinations have been experimentally compared. Conclusive evidence is therefore required to clarify the situation and allow informed decision-making. Research focusing on IRS/ITN

combinations should be initiated to answer several important questions regarding the need for such combined applications. In our view, the most important of these questions are: 1) whether the two methods complement or diminish beneficial effects of each other, 2) which insecticides are the most appropriate to use in co-applications, 3) what are the epidemiological and operational determinants necessary for optimal outcomes of such co-implementation, 4) whether co-application can be used to manage challenges like insecticide resistance and finally 5) how cost-effective would the strategy be.

Clearly these questions will require different kinds of studies. Therefore, research on combined ITN-IRS use should include: 1) experimental hut investigations where efficacies of the combinations are directly assessed against wild free-flying malaria vectors in malaria endemic areas, 2) mathematical simulations incorporating characteristics of candidate insecticidal applications to estimate likely benefits of the combinations in different scenarios, 3) long-term community-wide studies to determine effectiveness of the combinations and 4) cost benefit analyses of the combinations compared to individual methods on their own and also to other existing interventions. The proposed linkages between these studies are illustrated in Figure 2.

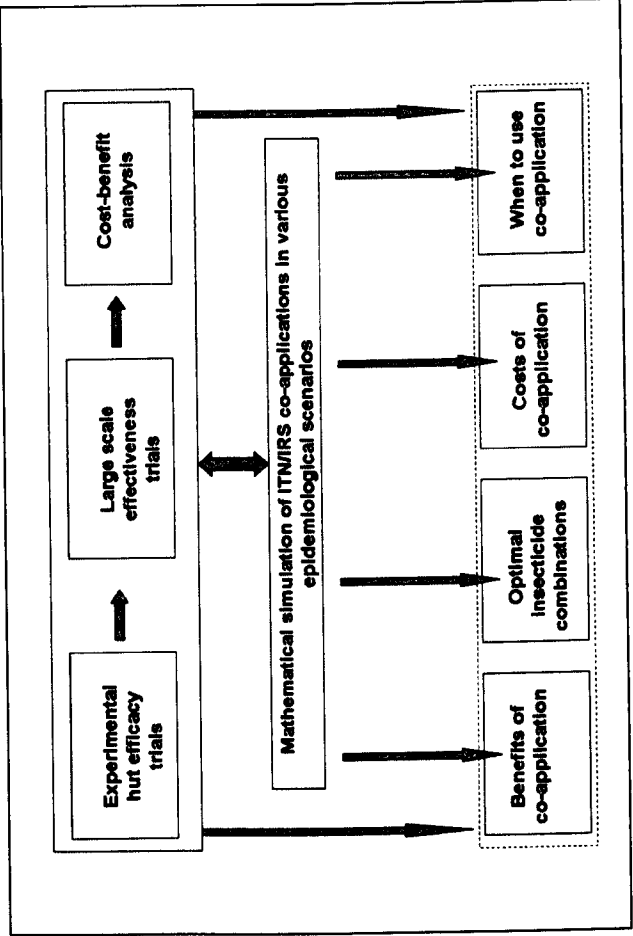


Figure 2: Conceptual sequence of research necessary to generate evidence for or against combined use of ITNs and IRS. From direct measurements in experimental hut trials, efficacious combinations of ITNs and IRS are identified and subjected to community wide effectiveness trials. Data from the effectiveness trials can then be used for cost benefit analyses. Where necessary, the mathematical models can utilize data from all the three studies (efficacy, effectiveness and cost-benefit analyses). Such simulations can: 1) help identify insecticides or combinations of insecticides for ITNs and IRS, which can then be re-evaluated in experimental huts, 2) help strengthen the design and implementation of new effectiveness trials and cost-benefit analyses and 3) enable extrapolation of information on efficacy and effectiveness of combined interventions in different epidemiological scenarios (including places with insecticide resistance). Results of these studies may then be examined to assess potential benefits of co-application, suitable insecticides for the combinations, and potential costs of the co applications as well as to determine when it is most appropriate to use the strategy

Discussion

As malaria control enters the phase of intensive and sustained vector control, health authorities must ensure that important gains so far achieved from existing interventions are not lost. Similarly, traditional control operations must shift dynamics to reflect the current goals of malaria elimination and eradication [9], and decisions guiding these interventions should be strengthened by incorporating locally generated evidence on effectiveness. ITNs and IRS, the most widely used malaria vector control methods, are already known to confer significant benefits against malaria [5, 8]. As correlations between these two methods and accrued health benefits become better understood, their acquisition and utilization also continue to expand requiring that the implementation is monitored closely to ensure proper use, optimal efficacy and maximum cost effectiveness, but also to prevent problems such as insecticide resistance and funding fatigue, as witnessed during the previous malaria eradication attempts of the 1950s and 60s [90]

The LLIN-IRS combination strategy is mostly recommended for accelerating control in high transmission areas [2, 12, 38, 41, 44], where either IRS alone or ITNs alone may not be adequate [41] yet transmission has to be reduced to near-undetectable levels to achieve any significant declines in malaria prevalence [41, 92-94]. However, ITNs and IRS can also be used together for different other reasons. With regards to household protection, the main reasons include ensuring protection where one of the interventions is weakened e.g. using LLINs where IRS activity decays after a short time [43, 46, 91] and providing additional level of protection e.g. by deterring mosquitoes from entering houses where people use toxic bed nets. However, with regards to community level protection, combinations may be used to increase overall coverage with vector control where complete coverage with only one

of the interventions is unfeasible throughout all endemic communities [43]. Besides, using IRS and LLINs with differing insecticides e.g. a pyrethroid-treated LLIN and the organophosphate or carbamate IRS may slow the spread of insecticide resistance, even though there is not yet any field evidence to support this possibility. As LLINs and IRS continue to be scaled up in malaria endemic areas, the threat of insecticide resistance also increases thus management of gene mutations to the common classes of insecticides (pyrethroids, organochlorides, carbamates and organophosphates) need to be emphasised. Given that this review considers data only from sites where no insecticide resistance had been reported, it is not possible to make inferences as to how combined insecticidal applications could work in areas with high insecticide resistance. Nevertheless, it is reasonable to assume that where insecticides of different modes of action are used, mosquitoes that are resistant to one of the insecticides could still be killed by the other insecticide, thus delaying any selection for resistant mutants among the mosquito populations. The actual possibility that combinations can remain effective even where vectors are resistant to one of the active ingredients should therefore be examined urgently, preferably by way of experimental hut studies.

In the process of writing this article, it became clear that even though combining ITNs and IRS is increasingly being practiced; there is insufficient evidence as to whether it is indeed better than ITNs or IRS on their own. The article explains how different insecticides can be combined to achieve maximum benefits at household level and how this can be translated to community level protection. For example, it is argued here that IRS and ITNs can complement each other at household level, for example where the IRS power decays rapidly or where the nets are torn and repeatedly washed. It is also inferred from synthesis of several previous studies that a higher level of reduction in exposure can be achieved if highly deterrent insecticides

such as DDT or lambda cyhalothrin are sprayed in houses where residents use nets treated with toxicants deltamethrin or alpha cypermethrin. The later argument is based on three principles: 1) that any insecticide can possess an array of properties which together determine its overall protective efficacy at household level, 2) that these properties function at different stages along the path of a mosquito approaching the human inside the house (Figure 1) and 3) that maximizing the protective benefits at each of these stages of action is an essential process in any attempt to optimize benefits obtainable from combined ITN-IRS interventions (Figure 1). It should however be noted that this argument is particularly true in areas where the vector is still sensitive to the insecticides, but that it may not hold true in DDT/pyrethroid resistance areas. Moreover, as a cautionary measure, DDT, which is the most common organochloride, is known to be affected by the same resistance mechanism that also affects pyrethroids, both classes being amenable to target-site resistance mediated by the *kdr* gene mutation [29, 36]. As such combination of DDT with pyrethroids must be very closely monitored given the likelihood of selection for more resistance without added benefit for protection. Generally, combination of pyrethroid-based IRS with any of the existing LLINs (all of which are also pyrethroid based) should be discouraged in places where there are any signs of emerging insecticide resistance, as this could lead to similar selection pressures.

Finally, to achieve community level effects, this paper recognizes the importance of coverage, i.e. proportion of all residents who consistently use these interventions, as a crucial factor. While toxic insecticidal interventions can kill large numbers of disease vectors thus contributing to mass communal benefits, it is also noted that interventions which deter mosquitoes from potential blood-hosts and indoor

resting sites also reduce the overall chances of these mosquito survival [85, 95], and malaria transmission if sufficiently high coverage is achieved [1, 6, 7, 20].

Conclusion and recommendations

It remains largely unclear whether using both ITNs and IRS would confer significant additional benefits relative to using either method alone. Even though there have been no specific studies that expressly tested this hypothesis, previous IRS and ITN trials and a number of mathematical models have resulted in mixed results showing improved benefits in some situations and redundancy in others. Nevertheless, there are still a number of reasons that theoretically justify combination of IRS and ITNs in households. For household level protection, it is strongly recommended that where residents use pyrethroid treated LLINs, the IRS product to be sprayed in houses to supplement the nets must be of completely different mode of action. The overall epidemiological outcome of such co-applications at community level would however depend on factors such as level of intervention coverage achieved, baseline epidemiological conditions, behaviour of malaria vectors, nature of insecticides used for IRS and the type of nets being used. Therefore, to maximize any possible additional benefits from IRS/ITN co-applications, rigorous field evidence, supported by mathematical modelling where necessary, should be pursued to support the entire process of decision making, including the selection of which insecticides to be used for IRS and what type of LLINs to use.

Author contributions

FO conducted the review and drafted the manuscript. Both FO and SM wrote the final version of the manuscript.

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Conflicts of interest

None

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PART TWO

Preview of Part Two

This part of the thesis consists of three chapters:

Chapter III: An experimental hut assay for evaluating long lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS). This chapter describes the development and baseline evaluation of an improved experimental hut design, which was then be used for evaluating candidate LLINs, IRS or combinations of the two as described in the next chapters of the thesis. This chapter is therefore mainly a methodological description but also includes results of baseline field experiments conducted to test and to optimize the experimental hut designs.

Chapter IV: Comparative evaluation of combinations of LLINs and IRS relative to either method alone. This chapter constitutes the main field study of this PhD research. It describes field experiments that were conducted to directly determine if indeed simaltenous use of LLINs and IRS in the same household can yield greater protection that the use of either method alone. The chapter provides results related to household level protection achievable with four different net types, three different IRS insecticides and a number of combinations of any of these nets and IRS.

Chapter V: Bio-efficacy and residual activity of insecticides used for LLINs and IRS: This chapter describes research conducted to complement studies in Chapter IV. Studies here included controlled bioassays performed to assess *how efficacious* and *for how long* the interventions tested Chapter IV would be against malaria vectors in the study area. It also included a series of insecticide susceptibility tests conducted on the local vector population, to provide indications on expectable level of insecticide efficacy. This way the studies enabled better interpretation of results from Chapter IV.

Important Note: Regarding the LLINs referred to in Chapters IV and V as **Icon Life® nets**, the supplier (Syngenta ltd) informed us at the end of our studies that this net type is the same as the one branded as **NetProtect®**, which has actually been given an interim approval by WHO (<http://www.who.int/whopes/quality/en>). However, in this thesis, the brand name **Icon Life®** has ben retained, given that this was the label on the actual nets that we evaluated in the studies described here.

Chapter III

A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: the Ifakara Experimental Huts *

Abstract

Differences between individual human houses can confound results of studies aimed at evaluating indoor vector control interventions such as insecticide treated nets (ITNs) and indoor residual insecticide spraying (IRS). Specially designed and standardised experimental huts have historically provided a solution to this challenge, with an added advantage that they can be fitted with special interception traps to sample entering or exiting mosquitoes. However, many of these experimental hut designs have a number of limitations, for example: 1) inability to sample mosquitoes on all sides of huts, 2) increased likelihood of live mosquitoes flying out of the huts, leaving mainly dead ones, 3) difficulties of cleaning the huts when a new insecticide is to be tested, and 4) the generally small size of the experimental huts, which can misrepresent actual local house sizes or airflow dynamics in the local houses. Here, we describe a modified experimental hut design - *The Ifakara Experimental Huts*- and explain how these huts can be used to more realistically monitor behavioural and physiological responses of wild, free-flying disease-transmitting mosquitoes, including the African malaria vectors of the species complexes *Anopheles gambiae* and *Anopheles funestus*, to indoor vector control-technologies including ITNs and IRS. Important characteristics of the Ifakara experimental huts include: 1) interception traps fitted onto eave spaces and windows, 2) use of eave *baffles* (panels that direct mosquito movement) to control exit of live mosquitoes through the eave spaces, 3) use of replaceable wall panels and ceilings, which allow safe insecticide disposal and reuse of the huts to test different insecticides in successive periods, 4) the kit format of the huts allowing portability and 5) an improved suite of entomological procedures to maximise data quality.

* Adapted from: Okumu F, Moore J, Mbeyela E, Sherlock M, Sangusangu R, Ligamba G, Russell T, Moore SJ: *A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: the Ifakara Experimental Huts*. PLoS ONE 2012, 7(2) e30967.

Background

To assess efficacies of house-hold mosquito control interventions, such as insecticide treated mosquito nets (ITNs) or indoor house spraying with residual insecticides (IRS), it is important to understand what happens to mosquitoes inside and around the dwellings in which these candidate interventions are located. Specifically, it is essential to know if the mosquitoes actually enter these huts, how long they spend inside the huts, whether they die inside the huts or after leaving the huts, and whether these mosquitoes successfully bite and take blood from persons inside these huts. The answers to all these questions represent efficacy of interventions against target mosquito species, and therefore influences the choices of vector control methods. Behavioural responses such as insecticide avoidance [1] and physiological events such as mosquito mortality, feeding or survival [1-3] are assessed and compared between houses with and houses without the intervention(s) being evaluated.

Difficulties associated with using local human houses to evaluate efficacy of vector control interventions

Ideally, trials of household vector control tools should be conducted in local houses, where the relevant interventions are intended for use. However, there are many variations between individual local houses, which can confound or even mask the real effects of candidate interventions being investigated. One common source of such variation is inconsistent number of house occupants and the associated differences in attractiveness of those occupants to host-seeking mosquitoes [4, 5], which means that even in the absence of any intervention, the number of mosquitoes entering any two different houses might be dramatically different. Another source of variation is type and texture of house construction materials. For example some huts may have mud

walls instead of plastered walls, while others may have thatched roofs instead of iron sheet covered roofs, creating different micro-climates indoors and subsequently differences in mosquito densities within these houses [6, 7]. Substrates used for house construction or for wall linings can also affect persistence of vector control insecticides sprayed on these surfaces [8, 9].

Third is the number and sizes of available openings in different houses, particularly where houses are poorly constructed. It is well-established that house design is a significant factor affecting mosquito entry into human houses and that screening of house openings, such as doors, windows and eave spaces can reduce both mosquito densities, and malaria cases in these households [10, 11]. The fourth important factor is spatial location of houses relative to mosquito larval habitats, which also affects the relative numbers of mosquitoes entering houses. This phenomenon has been observed in numerous studies where mosquito densities in houses near breeding habitats were significantly higher than houses further away from the known larval breeding sites [12-14].

Other than these inter-house differences, there are also difficulties related to mosquito collection procedures inside local human houses, as well as cultural issues that can also determine acceptability of such entomological procedures. For instance, houses often have items such as clothes, pictures or other assortments of objects hanging on walls, which can be hiding places for mosquitoes and potentially limit effects of insecticidal applications [15, 16]. Any attempt to remove these items, prior to testing indoor interventions would not only cause inconveniences to household members, but retaining them would also limit chances of recovering mosquitoes especially those that are killed as a result of the indoor interventions. The artefacts would also provide mosquitoes many un-standardised surfaces where they might rest

without being affected by a treatment, therefore biasing results. In some places it is culturally insensitive and considerably intrusive to collect mosquitoes in places such as people's bedrooms. Moreover, experience has shown that it can sometimes be mechanically impossible to fit standard mosquito traps onto windows or eaves of many of these houses without having to modify the openings or to minimise mosquito exit from cracks and holes on houses [17].

Early stage evaluations of most public health interventions require strict ethical guidelines to be followed [18]. Using experimental huts, occupied by volunteer adults who are fully informed of the risks and benefits associated with the study, therefore provides a way to avoid exposing the general public to any new interventions [19]. Besides, it can be very expensive to conduct proper large scale evaluations such as randomised controlled trials, which are the gold-standard for public health decision making and are designed to demonstrate direct relations between health benefits (e.g. reduction in disease prevalence or incidences) and the vector control intervention introduced [20, 21]. Also, given that causal chains in many public health interventions are inherently complex, and are constantly modified by a myriad of factors in space and time [20], RCTs often take extended periods of time to satisfactorily complete. Thus, experimental hut studies can also be useful in demonstrating causal relationships and also characterizing various biological indicators of health benefit, albeit at small scale, before an intervention is selected for RCT-style trials. For example, the huts can be used to directly observe and measure reductions in number of mosquitoes entering human occupied huts whenever an intervention is used inside that hut. Such an intermediate measurement, in this case reduced mosquito densities, can then be used to estimate likelihood of select interventions having epidemiological impacts at community level [22, 23]. Lastly,

small-scale experimental hut studies are considered as a cost-effective intermediate stage between laboratory and community trials to rapidly and safely select only those interventions with proven entomological impact, for further large scale epidemiological testing.

All the challenges outlined above highlight the need for specially designed huts constructed to enable representative monitoring and evaluation of household interventions against wild populations of disease-transmitting mosquitoes [24]. Other than collecting mosquitoes from inside surfaces like walls, ceilings and floors, the huts may also be fitted with special interception traps so that mosquitoes can be monitored as they enter and also as they exit huts. The experimental huts are usually standardised in size and shape and are sometimes constructed such that they look as similar as possible to the local houses in the study village [25]. This requires that in the beginning, a survey of local huts is conducted to identify important attributes such as shape, area of sleeping quarters, common construction materials, as well as size and number of openings like windows, doors and eave spaces (ventilation gaps under the roofs of many houses in the tropics). Cultural preferences including whether residents fit roof ceilings or window curtains should also be assessed.

A brief history of experimental huts and their applications in mosquito-related studies

In the early 1940s, Hadow *et al*, conducted a series of experiments involving mosquito collections inside local houses in western Kenya [26]. They quickly noted several differences between individual local houses in the same study area, and as a result of these observations, they created specially designed huts with standardised sizes and surfaces for purposes of mosquito collections. Important features of these early experimental huts were as follows: 1) they were similar in size and shape to the

local houses in the study area, 2) they all had exactly the same design so that it would be reasonable to compare mosquito catches between them, and 3) it was easy for persons to collect mosquitoes from all the inside surfaces of the huts, a requirement that was fulfilled by lining the inside walls with mud, covering the roof with a single-thickness hessian and using minimum furniture inside the huts. In addition, these experimental huts were windowless, had open eave spaces, tightly fitting doors and steeply pitched roofs to prevent rain draining inside. To attract mosquitoes, the Haddow *et al* huts were usually occupied by young local boys aged 10-12 years old [26].

After Haddow *et al* [26], several researchers began building on this work, leading to development of many early forms of experimental huts [24], including the mud-walled huts used by Muirhead-Thomson in Nigeria [27-30] and its modifications, later used by Burnett in mid 1950s [31] and by Hocking *et al* [32] to test residual insecticides against malaria vectors. Many improved hut designs appeared in the 1960s during the first malaria eradication era [24], including those used by Rapley and colleagues, which were suspended on concrete bricks and surrounded by water channels to prevent predator ants from climbing in and feeding on captive mosquitoes [33]. Unlike the early Haddow *et al* huts [26] that had been used primarily to catch mosquitoes resting indoors, these new huts were now fitted with traps on windows to also sample exiting mosquitoes. These improved huts, and other later designs, also fitted with window traps, are now commonly known as the window-type experimental huts [24].

In mid 1960s, a new type of experimental huts, referred to as veranda-type hut, was pioneered by Dr. Alec Smith working at the Tanzania Pesticide Research Institute (TPRI) in northern Tanzania [34, 35]. Smith's huts were different from

Rapley's huts in that other than having window traps on them, they were surrounded by screened verandas, in which mosquitoes were captured as they exited the huts. In experiments where a set of window traps were fitted to ordinary window-type huts and another set of window traps fitted onto veranda-type huts, leaving the verandas unscreened, it was concluded that presence of the verandas did not affect the total mosquito catches, nor the entry and egress patterns of mosquitoes [34].

Smith described the window-type experimental huts as being suitable for assessing mortality of malaria vectors, during evaluations of toxic insecticides but not evaluations of irritant insecticides, since mosquitoes irritated by insecticides would leave the huts earlier than normal and via any available opening including eave spaces. Such mosquitoes would thus go unaccounted for if window-type experimental huts were used [34]. He also noted that some non-malaria vector species such as *Mansonia uniformis* frequently exit huts through eaves as opposed to windows and are therefore best studied using veranda-type experimental huts rather than the window-type huts. Even then, the veranda-type hut itself did not completely solve this problem because of the way they are used; normally with two opposite verandas left open to let in mosquitoes, meaning that any mosquitoes exiting via eave spaces on these open sides still remain unaccounted for. This necessitated introduction of the inward and upward slanting barriers on top of the inside walls of veranda-type experimental huts: i.e. baffles that direct mosquito movement to allow mosquito entry but prevent exit. The barriers were originally truncated cones made of plastic mosquito gauze or wire mesh that slanted towards the apex of the roof at approximately 2cm away from but parallel to the roofing [36]. These slanting baffles allowed mosquitoes to enter the huts through the eave spaces but restricted their exit

through the same openings, even when highly irritant chemicals had been sprayed inside the huts [36].

At about the same time Hudson and Smith [37] developed another new hut with no verandas, but which instead was fitted with louvers angled at 53° so as to let in mosquitoes but minimise light that entered through the louvers. By attaching a window trap onto the east side of the hut, the mosquitoes were sampled while exiting towards the rising sun; and these catches multiplied by number of louvers so as to approximate total of mosquitoes entering the huts. This type of experimental hut was promoted mainly because it was simpler and cheaper to construct but also because it required simpler entomological collection methods [24, 37]. A recent modification of the louver hut is the west African design (also equivocally known as the “veranda trap hut”) developed at Institute Pierre Richet, in Côte d’Ivoire [38]. Mosquitoes enter these huts through louvers located on three sides and are trapped within the huts or in walled verandas fitted with a netted window located on the east side and closed with a drop cloth each morning.

Other more modern and innovative hut designs include the extraordinarily high Maya-style huts constructed by Grieco *et al*, to study behavioural responses of *An. vestitipennis* to insecticides in Belize [39]. These huts, had wooden plank walls and thatched roofs with apices rising as high as 4.5m from the floors, thereby requiring raised walk-ways, on which the person collecting mosquitoes would stand to inspect the high roof. These particular huts, like many earlier window-type experimental huts were also constructed in such a way that they could accommodate interception traps fitted on both windows and doors [39].

Most recently, portable wooden experimental huts have now been developed, which offer an added advantage of being easy to transport and to assemble onsite.

These portable huts were originally used by Dr. Nicole Achee and colleagues in Belize, Central America, to recapture marked mosquitoes released at different distances [25]. With regard to construction materials and also dimensions of sleeping quarters, these huts were comparable to local village huts in the study area, in the central Cayo district of Belize. Portability was introduced by using a collapsible aluminium framework, allowing the collapse of the entire superstructure of the huts (including roof, gables and walls) by simply unbolting the metal bars in the framework. Furthermore, both the roof and the hut walls could be dismantled into 4 hinged units and 16 planks respectively, for loading onto transporter-trucks [25].

Here, we describe a new improved hut type, The Ifakara experimental hut, which encompasses several essential properties of the previous hut designs.

Methods

The Ifakara experimental huts

Design, general characteristics and dimensions

The Ifakara experimental huts are a new kind of hut, recently developed at the Ifakara Health Institute, Tanzania. The hut design encompasses proven merits of previous huts, but also aims to minimize some disadvantages associated with those previous designs. First constructed in 2007, these huts are already being used in Tanzania, Kenya, Zambia and Benin for various studies, including evaluation of LLINs and IRS (Okumu *et al* Unpublished), house screening against mosquitoes [40], mosquito repellents (Ogoma *et al* Unpublished), synthetic mosquito attractants [41] and mosquito killing fungal pathogens [42].

The original design of these huts was created to incorporate the portability principles earlier described by Achee *et al.*, [25]. However, with regard to shape,

average dimensions and inside surface linings, the Ifakara experimental huts are similar to local village houses in rural communities in south eastern Tanzania, where these huts were originally used (Figure 1). It had been directly observed that local houses in Tanzania were mainly mud or brick walled, with thatched roofs [43]. However over the past three years, the proportion of roofs constructed from iron-sheet has increased to almost half [44]. Specific hut dimensions were collected using a housing survey in the study village.

The framework and detailed dimensions, as well as important construction stages, leading up to a finished Ifakara experimental hut, are shown in Figures 2 and 3. When completed, each hut covers a floor area 6.5m in length by 3.5m wide inside with a 50cm walkway around the outside of the hut, and rises 2.0m on the sides and 2.5m to the apex of the roof. The huts have galvanized iron frames, with roofs made of corrugated iron sheets, which are overlaid with thatch to ensure that indoor temperatures do not exceed the average temperatures inside local village houses (Table 1). The walls are constructed using canvas on the outside but are lined on the inside using removable wood panels that are coated with clay mud, which was the most common wall construction material used and found locally in the study area (Figures 1 and 3). The inside surfaces of the roofs are lined with woven grass mats, locally known as *mikeka*, and which also were common materials that local people use to make ceilings. Each hut has four windows (two on the front side and two on the back side) and one door (on the front side). For ease of transport and assembly on-site, the huts are designed and constructed in kit-format, with all individual pieces made in standardized sizes. Therefore despite the relatively large size, it takes approximately 1–2 days to complete assembling one hut at the field site.



Figure 1: A typical local house used by communities in southern Tanzania, where the Ifakara experimental huts were first tested.

Features to prevent contamination when working with insecticides

To ensure that the main framework of the hut is never contaminated by any chemicals that may be used inside the huts or sprayed on the walls and ceilings (for instance when evaluating indoor house spraying with residual insecticides), continuous sheets of polyethylene (PE) are tightly fitted in the space between the outer framework of the huts and the mud panels and *mikeka* ceilings, which make up the insides hut surfaces. This PE sheeting, together with the mud panels and the *mikeka* ceiling, are not permanent components of the huts, and can be replaced whenever a new intervention or insecticide is to be tested in these experimental huts. The old materials can then be safely disposed of by incineration $>1000^{\circ}\text{C}$ using a T300 trench air burner (Air Burners LLC, FL, USA) available at the Ifakara Health Institute. Each Ifakara experimental hut has one door, four windows and an open eave space all round (Figures 2 and 3).

Features to prevent predation

To prevent scavenger ants from eating captive mosquitoes, the huts are suspended above ground using pedestals standing on water-filled metallic bowls (Figure 2D). The water in these bowls is regularly replenished and sprinkled with used-oil to also prevent mosquito breeding in them. Other than these measures, additional anti-ant precautions include regular cleaning of the huts, removal of shoes whenever one goes into the huts and clearing of all vegetation near and under the huts, which might otherwise be used by ants as a means to climb onto the huts (Figure 3D).

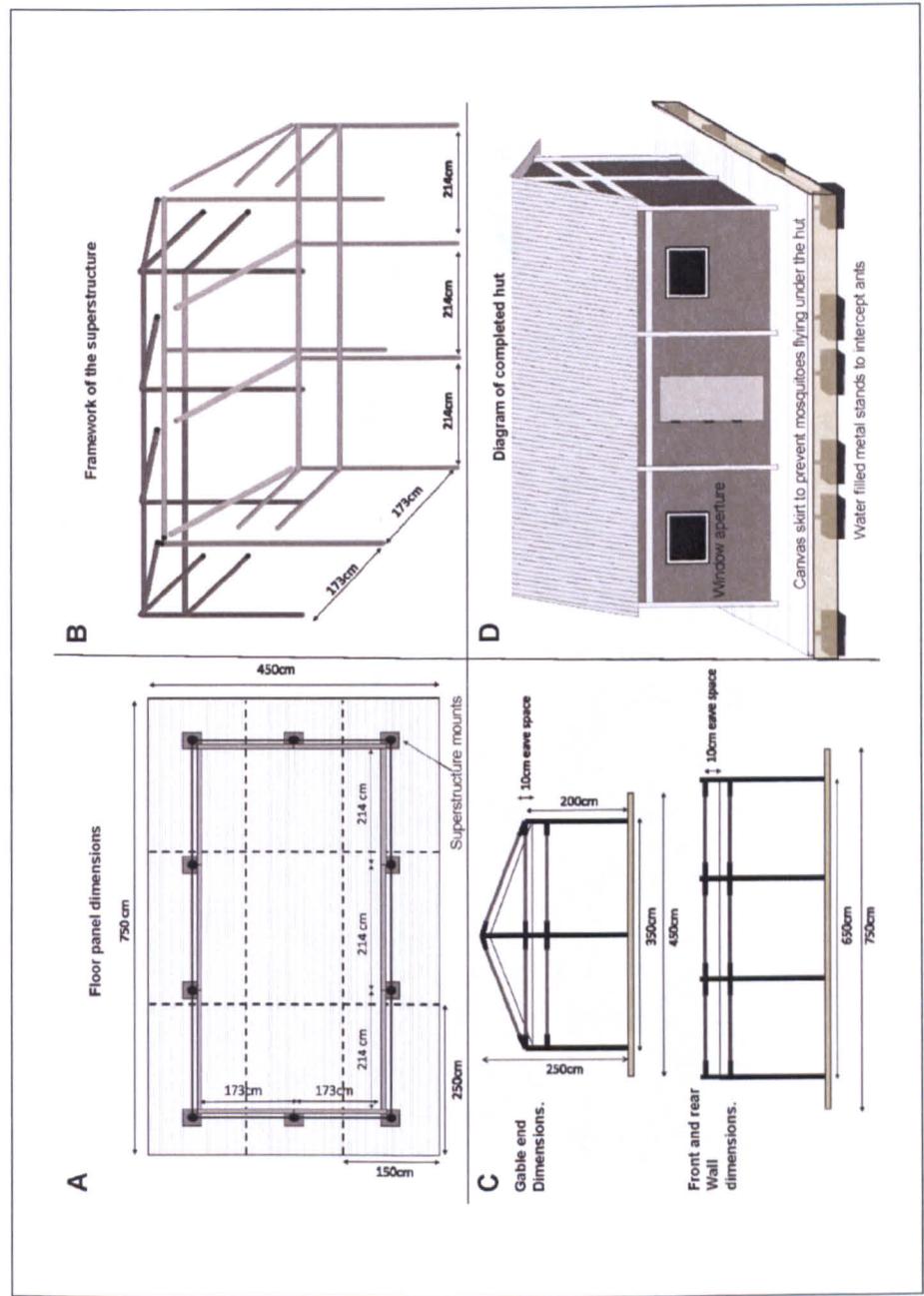


Figure 2: Diagrammatic representations of: the floor plan (panel A), the framework of the superstructure (panel B), side plans (panel C) and a complete view of the Ifakara experimental huts (panel D), showing important features.

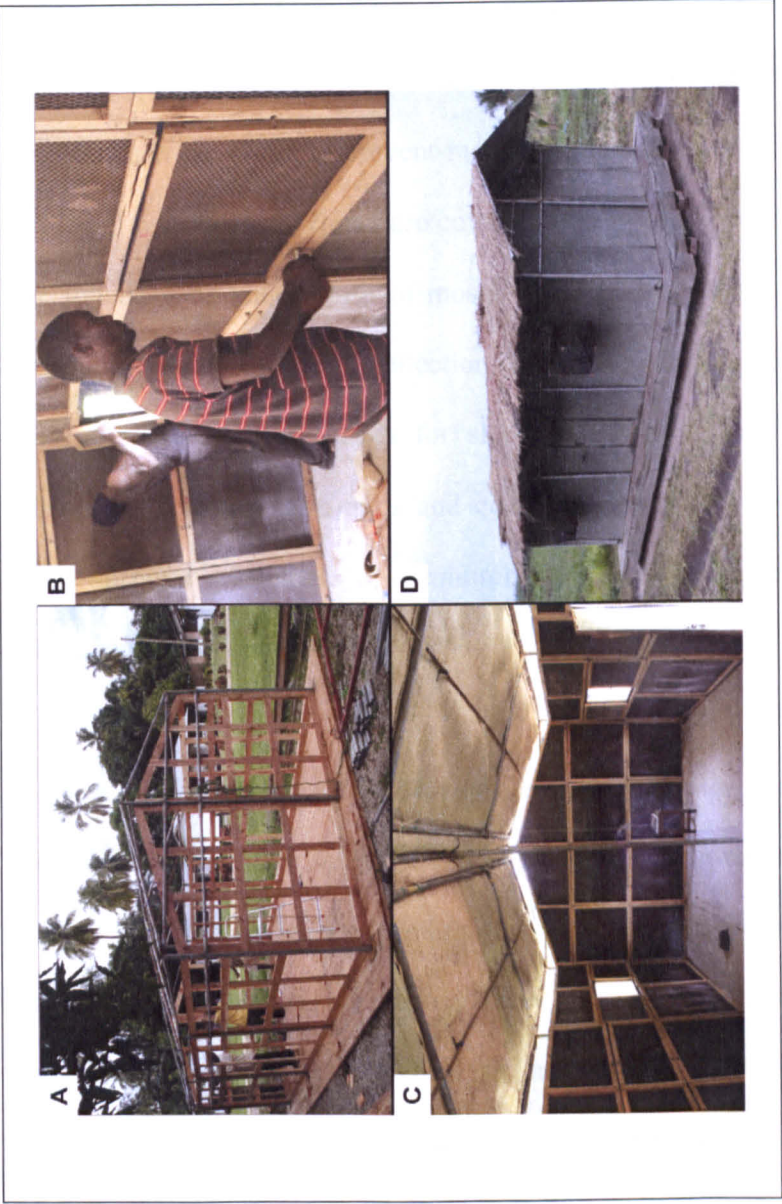


Figure 3: Pictorial representations of selected steps in the construction of the Ifakara experimental huts. Panel A shows the main framework of the Ifakara experimental huts under construction at the workshop. Panel B shows technicians fitting the wall panels, (which are made of chicken wire on wooden frames), onto the inside walls of the Ifakara experimental huts. Panel C shows the inside walls of the huts after fitting the chicken wire wall panels and also the palm woven (*mikeka*) ceiling on the underside of the roof, but before the inside walls are covered with mud, and Panel D shows a completed and functional Ifakara experimental hut, fitted with interception traps on windows and eave spaces. It should be noted that the overall shape and dimensions are set to match the typical local houses, shown in Figure 1. The hut is suspended on water-filled metal bowls to prevent predator ants, which would otherwise prey on the trapped mosquitoes.

Features to prevent loss of mosquitoes

The huts are tightly finished and all individual pieces are well fitting, so that the only points for mosquito escape are windows and eave spaces, where interception mosquito traps are fitted. Any unwanted gaps around doors, eaves and windows are filled with hardened foam, to prevent mosquitoes that have entered the huts from escaping unaccounted for. As an additional precaution an oversized curtain can be hung on each the doors to prevent mosquito movement through the doors in case of accidental opening. The floors are covered with white, wipe-clean linoleum to ensure that any dead or knocked-down mosquitoes can be easily recovered. To minimize obstruction during mosquito collection, only the minimum essential furniture is kept inside the huts, i.e. two beds for sleeping volunteers and a ladder used during collections from the eave traps and ceilings. This practice, together with the lined inside surfaces and floors also minimize potential mosquito hiding places in the Ifakara experimental huts.

Traps and baffles used on the Ifakara experimental huts

The huts are fitted with interception traps both on windows and eave spaces to catch mosquitoes. The designs and dimensions of these interception traps are illustrated in Figure 4. The versions presented here are the final result of a gradual trap development and improvement process, and should be considered as accessories of the Ifakara experimental huts, rather than as independent mosquito sampling tools. These traps can be fitted facing the inside of the hut to catch entering mosquitoes (in which case they are referred to as entry traps), or facing the outside so as to catch exiting mosquitoes (in which case they are referred to as exit traps). The entry and exit traps are specially designed to fit onto either windows (i.e. window traps) or on

the eaves of the huts (i.e. eave traps), as depicted in Figures 3D and 4. In practice, the eave exit traps are therefore physically the same as eave entry traps, while the window exit traps are also physically the same as window entry traps. The traps are made of ultraviolet resistant shade netting (TenTex polypropylene net), mounted on a 5mm wire frame, which is joined together using wooden blocks. The front end of each trap has a letterbox-shaped opening (measuring 80cm by 3cm on the eave traps and 40cm by 3cm on the window traps), to ensure that mosquitoes passing through the eave spaces or windows are let into the traps easily, but that these mosquitoes, once inside the traps cannot leave the traps as easily (Figure 4). To enable attaching onto the experimental huts, the netting with which the traps are made is extended to form attachment flaps specially fitted with Velcro-lined double seams. The frames of both window and eave spaces on all huts also have Velcro linings, so that the traps can be attached onto them. In this hut design, no traps are fitted onto the doorways, which instead are mostly kept shut except during passage of personnel. Moreover, we ensured that all the door shutters were tightly fitting and that there were no open spaces through which any mosquitoes could fly in or out. As such the only entry and exit points available for the mosquitoes were the eave spaces and windows.

Baffles on the other hand consist of upward-slanting and inward-facing netting barriers that are fitted on top of the walls of the experimental huts, so as to allow in mosquitoes, while at the same time preventing those mosquitoes that are already inside the huts from exiting via the same spaces (Figure 5). Netting was selected to encourage dispersal of human odour from the huts and therefore to maximise mosquito attraction to the huts [45]. The positions of the baffles on the eave space are interspaced between exit traps such that all mosquitoes that enter the huts can exit only via those spaces fitted with the exit traps (Figure 5C&D). The concept of

interspacing baffles with exit traps all round the eaves also ensures that, similar to local human houses, there are adequate spaces through which mosquitoes can enter the experimental huts. It is expected that this practice removes directional bias, allows kairomones from human volunteers to be dispersed in a plume similar to that from a local house and maximises the spaces available for mosquito entry to maximise numbers in the huts. This is desirable in many field experiments involving free-flying wild mosquito populations, especially in areas where mosquito numbers are low, to improve the discriminatory power of the experiments. The baffles slant towards the apex of the huts and are held in parallel to the roofing using thin metal hooks (Figure 5B&C). There are two different sizes of these baffles, designed to fit onto either the gable side of the huts (175cm by 50cm baffles) or onto the long (front and back) sides of the huts (120cm by 60cm baffles). All baffles have Velcro-seamed ‘wing’ flaps, with which they are affixed to the roofs or walls of the huts, so that mosquitoes do not escape through the sides (Figure 5A&B).

In addition to mosquito collections using the interception traps, mosquitoes that enter the huts but fail to exit (e.g. fed mosquitoes resting indoors or those mosquitoes that are killed or knocked-down by insecticidal interventions) can be retrieved by direct indoor collections, from hut walls, ceilings or floors, using mouth aspirators. This procedure was implemented in the experiments conducted to test the experimental huts, as described later in this article.

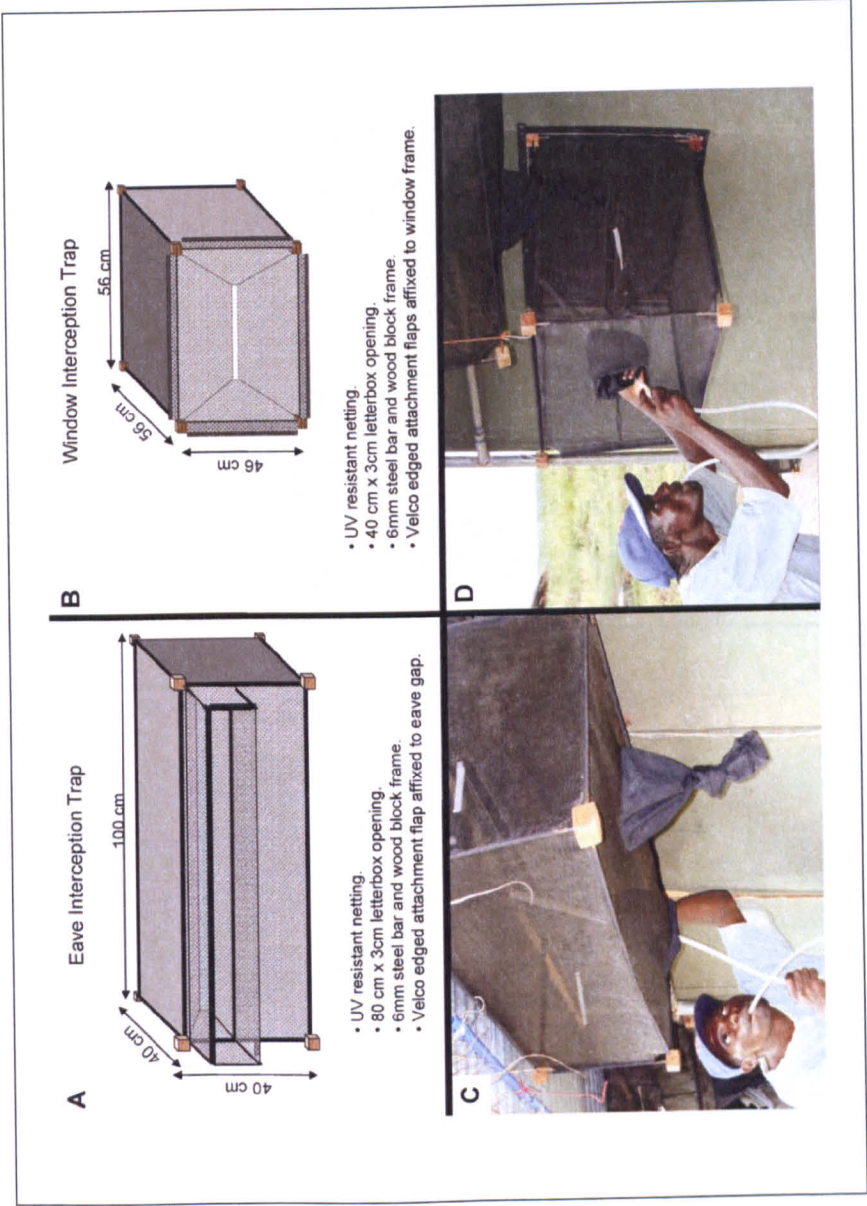


Figure 4: Diagrammatic illustration of eave trap and window trap. Panel A and B shows the dimensions and materials used to construct these traps, while panel C and D shows the eave and window traps fitted onto an Ifakara experimental hut during collection.

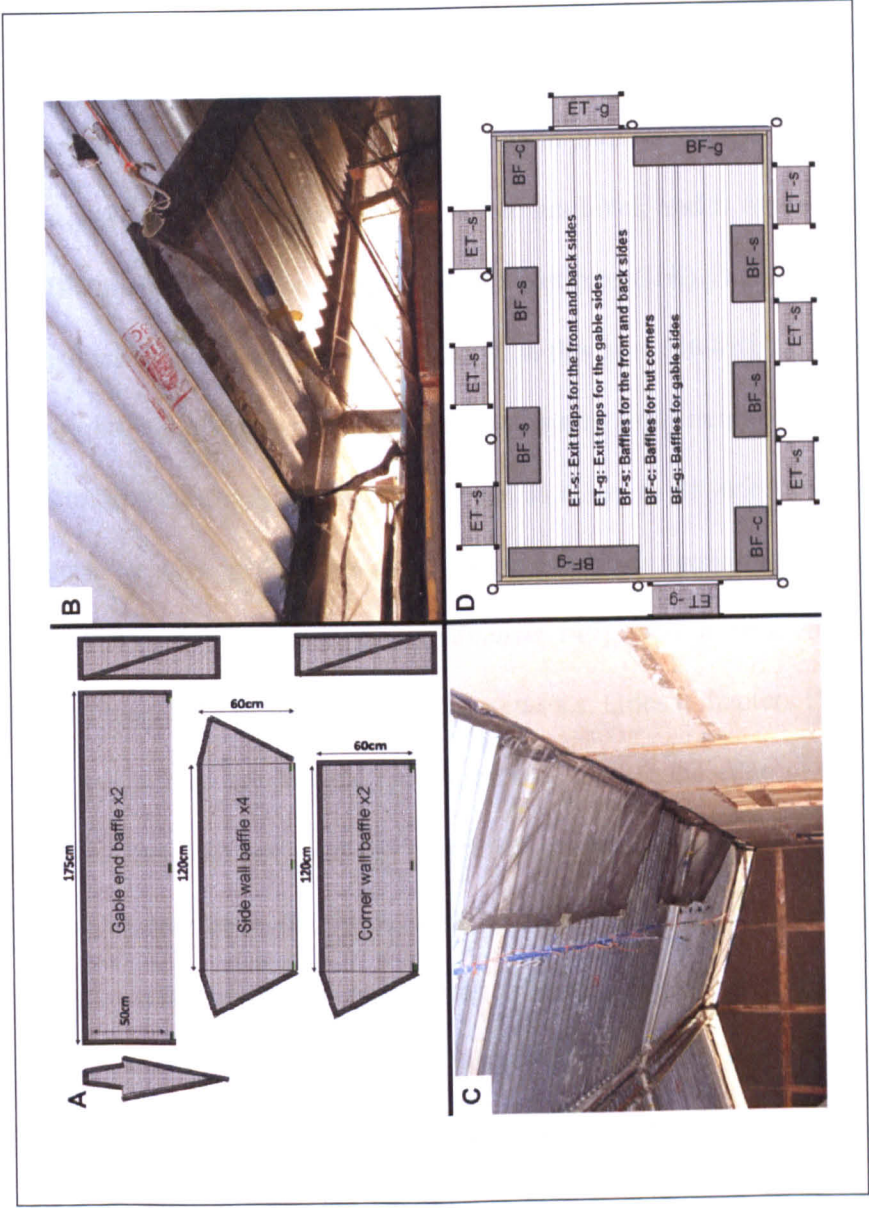


Figure 5: Netting baffles used in the Ifakara experimental huts: Panel A shows the design and dimensions of the different baffles used on front, back and gable sides, panel B and C are pictures showing two baffles fitted inside the huts and panel D shows the general layout of the baffles as interspaced with exit traps. Note that even though this diagram shows no *mika* ceiling under the roofs, the ceiling is an essential feature of all completed Ifakara experimental huts as shown in Figure 3

Geographical positioning of the Ifakara experimental huts within the study area

To exemplify how best to spatially position these experimental huts during entomological studies, this section describes geographical sitting of nine Ifakara experimental huts, relative to the positions of local human houses in a rice growing village, in south eastern Tanzania, where we evaluated insecticide treated nets (ITNs) and indoor house spraying with residual insecticides (IRS) between 2009 and 2011 (Chapters IV and V). The study site was in Lupiro Village (8.385°S and 36.670°E), Ulanga District. It lies 300 meters above sea level, and is approximately 26km south of Ifakara town, where Ifakara Health Institute (IHI) is located. Although malaria transmission has been reducing steadily in this area [46-48], residents still experience perennially high transmission; latest estimates from neighbouring villages showing that unprotected individuals can still get as many as 81 infectious bites per year [46]. Malaria vectors in the area comprise primarily *An. gambiae* complex species, more than 95% of which are *An. arabiensis* [49], and a few *An. funestus* complex mosquitoes, 99% of which are *An. funestus* s.s. Giles (Chapters IV and V).

The huts are located on a stretch of land at the edge of the village, such that that the huts are between the perennial irrigated rice fields (being the main larval mosquito habitat in the study area) and human settlements (Figure 6). For newly-emerged mosquitoes, this positioning enhances accessibility of these huts, relative to local houses. Considering natural dispersal patterns of mosquitoes over landscapes, and associated heterogeneities of their population densities [13, 14], it was envisaged that emergent host-seeking vectors from the irrigated rice fields are invariably more likely to first encounter these experimental huts, than the residential village houses, which are geographically farther from the breeding sites (Figure 6). Also, one other advantage of this positioning strategy is that even though our studies often involve

large groups of volunteers and field assistants working in the huts at night, there is minimal disturbance to local villagers, since the huts are far from the main settlement area.

Climatic factors inside and outside the Ifakara experimental huts

To monitor the various climatic variables that may affect densities and/or behaviour of mosquitoes in the study site, an electronic weather station (LaCrosse Technology, USA) was positioned at the site, with an indoor sensor located inside one of the experimental huts. Using this wireless station, climatic variations were continuously recorded both indoors and outdoors on an hourly basis. These included indoor and outdoor temperatures and relative humidity but also wind speeds, wind direction, and rainfall. In addition, a set of portable data loggers (Tinytag Plus, TGP-4500) were introduced in two experimental huts and two local huts (one having a grass thatched roofing while the other having iron sheet roofing), so temperature and humidity changes could be directly compared between the hut types.

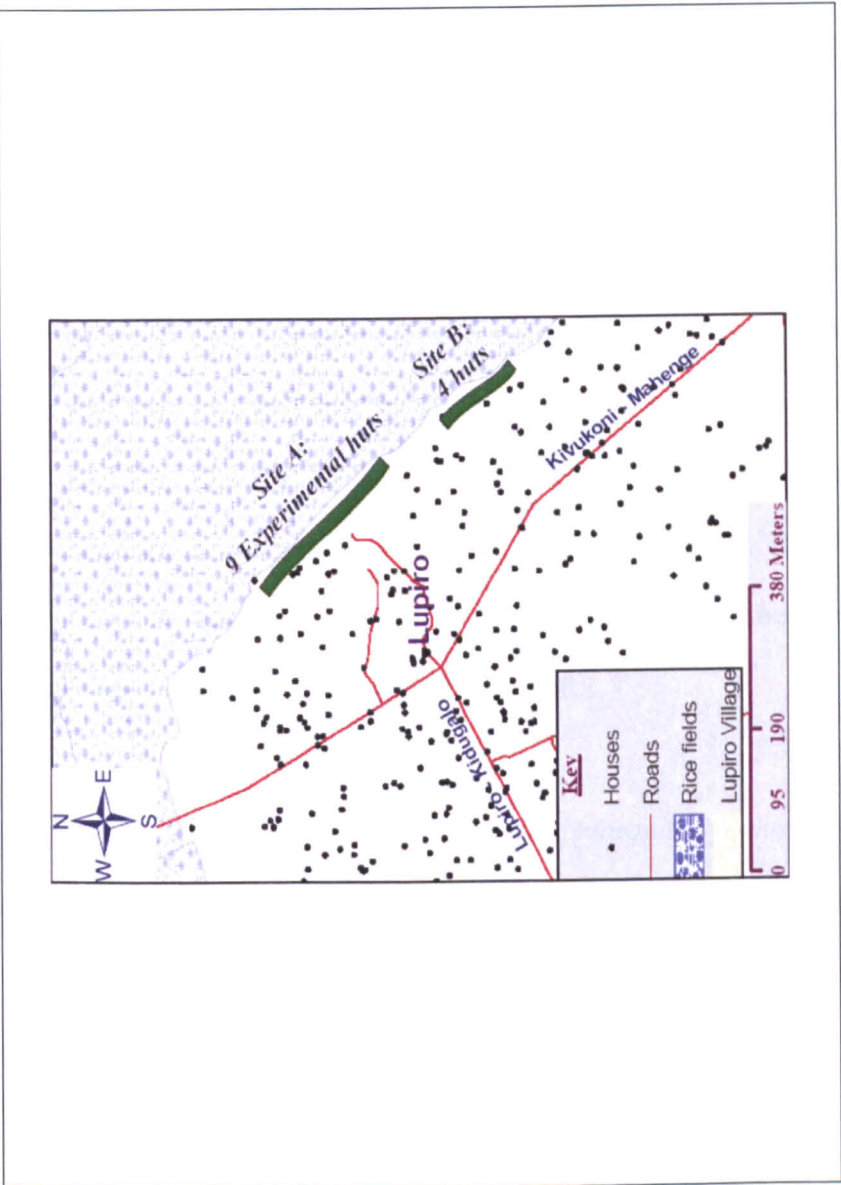


Figure 6: A map of the study area showing two sites at the edge of the village where Ifakara experimental huts are currently located. Site A has 9 huts while site B has 4.

Baseline studies using the Ifakara experimental huts: assessment of natural behaviour of mosquitoes in and around human occupied huts, and evaluation of a natural spatial repellent sprayed in the huts

Prior to testing any vector control technologies using the Ifakara experimental huts, studies were performed to understand how local mosquito vectors in the study area naturally behave in and around human occupied huts. It was also necessary to assess efficacies of both the baffles and the interception traps, as used on Ifakara experimental huts. The interception traps were evaluated in comparison to a standard entomological sampling method for indoor host-seeking mosquitoes, the Centres for Disease Control Light Traps (CDC-LT), set near a human volunteer sleeping under a bed net [50, 51]. This validation of efficacy of baffles and interception traps was performed using four experimental huts as described below. These initial studies also enabled us to trouble-shoot and to assess the utility of these huts for evaluating insecticidal applications such as LLINs and IRS.

Studies to determine: a) the times when local mosquito species normally enter human occupied huts, and b) the efficacy of entry traps relative to the standard, CDC-Light Traps

Four Ifakara experimental huts, each with two volunteers sleeping under non-insecticidal bed nets, were used. The four huts were paired, and in each pair one of the huts was fitted with entry traps on windows and on eave spaces, while the second hut had CDC-LT set up at a position between the two human volunteers sleeping under non-insecticidal bed nets, to catch mosquitoes entering the huts [51, 52]. The CDC-LT was fitted with timed bottle rotator (John Hock, FL, USA) to sample mosquitoes every hour. The volunteers stayed inside each hut between 7pm and 7am, during

which time the traps were emptied each hour and all mosquitoes collected were aspirated into different paper cups, clearly labelled to show both the time of collection and type of traps used. Every night, the entry traps and the CDC-LT were rotated between individual huts in each pair of experimental huts. These cross-over tests were replicated 8 times over a period of 16 consecutive nights and each morning, all mosquitoes collected were sorted by taxa and their respective counts recorded.

Studies to determine: a) times when local mosquito species normally exit houses, b) efficacy of the exit traps and c) efficacy of the baffles fitted on open eave spaces of the Ifakara experimental huts.

Four experimental huts, each with 2 volunteers sleeping under untreated bed nets, were used. On two of the huts, exit traps were fitted on 2 windows facing east with the other 2 windows open to allow mosquitoes to enter. Exit traps were also affixed to the eave spaces, interspaced with one-meter open spaces between them, as shown in Figure 5C, to allow mosquitoes to enter huts via the eaves. As a standard, CDC-LT was set inside the remaining 2 experimental huts [51, 52]. Since we also wanted to assess whether our baffles can indeed minimize possibility of mosquitoes exiting directly through the open eave spaces as opposed to flying into the exit traps themselves (Figure 5), two of the huts (one with exit traps and another with CDC-LT), were additionally fitted with the baffles.

The four treatments tested each night were therefore as follows: Treatment 1) one hut fitted with baffles and exit traps; Treatment 2) one hut fitted with baffles and CDC-LT; Treatment 3) one hut fitted with no baffles but with exit traps; Treatment 4) one hut fitted with no baffle but with CDC-LT. These treatments were rotated between huts on nightly basis, and were compared against each other in a 4 x 4 Latin

square experimental design with each round replicated 4 times over a period of 16 consecutive nights. This experiment was repeated twice at different times. The volunteers stayed indoors between 7pm and 7am each night, and mosquitoes entering the huts were sampled hourly using the exit traps or the CDC-LT that was fitted with a timed CDC-bottle rotator (John Hock, FL, USA). The collected mosquitoes were aspirated into different paper cups, clearly labelled to show both the time of collection and type of traps used. Each morning, all the mosquitoes were sorted by taxa and their respective counts recorded.

Studies to: a) determine whether it is more efficacious to use both exit and entry traps on each experimental hut, relative to using just one trap type on the huts, and b) compare the number of mosquitoes entering the individual huts.

We initially envisaged that by sampling exiting and entering mosquitoes in any given hut during the same night, we would significantly reduce potential biases possibly arising from daily variations of mosquito densities as well as wind direction. An experiment was therefore conducted in which individual experimental huts were fitted with either a combination of entry and exit traps, or with just entry traps alone or exit traps alone. Since this experiment involved mosquito collections in all the 9 experimental huts earmarked for our subsequent studies, it also enabled us to assess if there were any differences in numbers of mosquitoes entering the different individual huts in their designated locations.

Tests were conducted as follows: nine experimental huts were used, each with two volunteers sleeping under non-insecticidal bed nets. Each night, three of the nine experimental huts were fitted with a mixture of entry and exit traps (Treatment 1), another three were fitted with entry traps only (Treatment 2) and the remaining three

fitted with just exit traps only (Treatment 3). Whenever the exit traps were used, and also whenever a mixture of entry and exit traps were used, baffles were fitted on the open eave spaces to prevent mosquitoes from exiting the huts via spaces other than those fitted with exit traps (Figure 5). In the three huts with mixtures of the entry and exit traps, the different trap types were interspaced so that any two opposite sides of the huts had equal number of entry traps or exit traps.

The trap arrangements were rotated weekly in such a way that at the end of the 3-week experiment, each hut had been fitted with each arrangement for one week (working for six nights a week). Due to logistical difficulties, the entry and exit traps were emptied three times a night at 11.pm, 3.00am and 7.00am, as opposed to hourly as in the previous experiments. To ensure that the total number of mosquitoes entering each hut was accounted for, further collections were conducted each morning from the inside hut surfaces using mouth aspirators, to retrieve any mosquitoes that had entered the huts during the night but failed to exit. The mosquitoes collected from each hut were aspirated into different paper cups, clearly labelled to show time of collection, trap from which the mosquitoes originated and trap arrangement used on the hut. Each morning, the mosquitoes were sorted by taxa and their respective counts recorded.

Studies to troubleshoot and optimize operations involving application of insecticides in the Ifakara experimental huts

Prior to introduction of any insecticidal applications in these huts, studies were conducted in which a behaviourally active test compound was applied on the mud panels of the experimental huts (Figure 3). A botanical mosquito repellent, para-methane 3,8 diol (PMD), which does not have long-term residual effects, was selected for this purpose [53, 54]. The low-residual property was particularly important so that

the test compound would not confound effects of any other insecticidal applications used in the experimental huts at a later date.

This step enabled us to identify any potential limitations of the huts and vital adjustments necessary, meaning it was essentially a troubleshooting and optimization process, with a secondary objective of evaluating effects of PMD on behaviour of local mosquitoes. Specific activities that required trouble shooting included, spraying techniques, hourly mosquito collection, data management techniques, ways of addressing important volunteer needs, and other minor logistical challenges such as dealing with accidental scavenger-ant invasion in the experimental huts.

Four experimental huts each with 2 volunteers sleeping under untreated bed nets were used. Two of the selected huts were treated with PMD at a concentration of 1gm^{-2} sprayed on the hut walls. PMD is not typically sprayed on walls so the concentration was based on laboratory data of relative repellency compared to DDT as a standard (Dr. John Grieco, personal communication). Once the target doses of PMD were calculated, the total amount of PMD required per hut was weighed and thoroughly diluted in the correct volume of water predetermined to cover the entire internal wall surfaces of the huts. The spraying was performed using standard Hudson Expert™ sprayers as illustrated in Figure 7. The other 2 huts were left as controls and were sprayed with only water. The four experimental huts were paired so that each pair had a PMD sprayed hut and a control hut to be directly compared against each other in two cross-over experiments as follows: Huts in the first pair were fitted with entry traps on windows and eaves to catch mosquitoes while entering huts. On the other hand, huts in the second pair were fitted with exit traps on windows and eaves to catch mosquitoes while leaving the huts. Baffles were added in the second pair of huts to limit unmonitored mosquito exit through the eave spaces. None of the treated huts

was re-sprayed during the entire experiment period, which lasted 6 nights. Given the said purpose of this experiment, we did not conduct any assays to determine residual content of the PMD on the sprayed walls, hence the experimental period was limited to only six nights rather than several weeks as is common practice in experimental hut evaluations of public health insecticidal applications [19].

Each night, the sleeping volunteers rotated between the two huts in each treatment pair of huts to eliminate potential confounding effects resulting from any differential attractiveness of volunteers to mosquitoes [4, 5]. The exit and entry traps were emptied hourly from 7pm to 7am and the collected mosquitoes from each hut were aspirated into different paper cups, clearly labelled to show the time of collection, the trap from which the mosquitoes originated and whether the experimental hut had been sprayed with PMD or not. In addition, to ensure that the total number of mosquitoes entering each hut was accounted for, further collections were conducted each morning from the inside hut surfaces using mouth aspirators, to retrieve any mosquitoes that had entered the huts during the night but failed to exit.



Figure 7: Picture of a fully suited spray person applying PMD onto inside walls of the Ifakara experimental huts using a standard Expert Hudson™ sprayer

Identification of mosquitoes

Each morning, all mosquitoes were sorted by taxa and the respective counts recorded. Malaria vectors, *An. gambiae* complex and *An. funestus* complex mosquitoes, and other *Anopheles* mosquitoes were first distinguished morphologically from Culicine mosquitoes of other genera found in the study area i.e. *Culex* species and *Mansonia* species [55]. Molecular analysis by way of multiplex polymerase chain reaction (PCR) [56], was then used to distinguish between *An. arabiensis* and *An. gambiae* s.s, the most predominant members of the *An. gambiae* complex found in the study area. Although, no PCR analysis was done on *An. funestus* complex mosquitoes collected during these early studies, the procedure was later incorporated in our subsequent tests, where all mosquitoes in this complex were shown to be *An. funestus* s.s [57].

Data analysis

Data analysis was performed using SPSS version 16 (SPSS Inc. Chicago, USA). Data were analysed with generalized linear models with a negative binomial distribution and a log link to account for the over-dispersed nature of mosquito count data. Since most of the experimental huts data was clustered in individual huts, between which different treatments were rotated in a complete randomized block design, hut was included as a factor variable in all analyses. All models contained an intercept. Robust standard errors were used to account for any correlation between observations within huts. When comparing mosquito catches related to any two categories (e.g. eaves trap vs. CDC-LT, or PMD sprayed hut vs. unsprayed hut), the regression intercepts were calculated and then exponentiated (as data were on a log scale) so as to enable the determination of efficiency of one treatment relative to an

indicator variable reference, normally the control. Effects of the PMD spray was estimated following the WHO standard methodology [19], as a percentage reduction in number of mosquitoes caught in the PMD sprayed huts relative to the number of mosquitoes caught in the control huts.

Protection of participants and ethics statement

Participation in all our hut studies was entirely voluntary and the volunteers could leave at will at any stage during the experiment. After full explanation of purpose and requirements of the studies, written informed consent was sought from each volunteer prior to the start of all experiments. All participants received nightly wages as an incentive and to compensate for their time. Only males over 18 years were recruited as there are cultural implications of women working at night, and also ethical implications of recruiting women of childbearing age to a study where malaria infection could occur. Volunteers sleeping inside Ifakara experimental huts use intact bed nets so as to prevent mosquito bites. This is a minimum acceptable protection for research conducted in studies involving wild, potentially infectious mosquitoes, and was used in all cases as the universal experimental control when evaluating any candidate insecticidal applications. The volunteers were also provided with access to weekly diagnosis for malaria parasites using rapid diagnostic test kits and treatment with the first-line malaria drug (artemether-lumefantrine) in case they contracted malaria. Fortunately, none of the volunteers became ill during the period of these experiments. The study was approved by the Institutional Review Board of the Ifakara Health Institute (IHRDC/IRB/No.A019), the Tanzania National Institute of Medical Research (NIMR/HQ/R.8aNo1.W710) and the London School of Hygiene and Tropical Medicine (Ethics Clearance No. 5552).

Results

Climate measurements inside and outside Ifakara experimental huts and local houses

Table 1 shows mean indoor temperatures and relative humidity in both the Ifakara experimental huts and local houses in the study area. Indoor temperatures were similar between the experimental huts and the local grass thatched houses both during the day and during the night. One way analysis of variance revealed no difference in indoor night temperatures ($F=0.069$, $DF=2$, $P=0.998$) between the huts, but day-time temperatures were higher in local iron-roofed huts than in both the experimental huts and local grass-thatched huts ($P<0.001$). There was a significant difference in relative humidity between local iron roofed huts and the experimental huts ($F=4.520$, $DF=2$, $P<0.001$), but not between the experimental huts and local grass thatched huts.

Tables 2-3 provide a summary of climatic data at different times in 2010. As depicted by the standard deviations in Table 2, it is evident that for all of the important climatic factors, there were large variations during the daytime, but only minimal variations at night, when most of the mosquito collections were done. Also, we observed that even though it was warmer outdoors than indoors at daytime (average temperatures of 28°C versus 26°C), the huts were warmer than the outdoor environment at night (average temperatures of 23°C indoors versus 21°C outdoors). Similarly it was always more humid inside the huts than outside during the day (mean relative humidity of 66% versus 62% outdoors), but this was reversed during the nights, when it became more humid outdoors than indoors (mean relative humidity of 68% versus 84% outdoors). Finally, we also observed that winds were stronger and more variable during the day than at night, during which times the air was almost still (Table 3).

Table 1: Mean and standard deviations (SD) of daily temperatures and relative humidity (%) inside Ifakara experimental huts, as compared to local huts that have either grass thatched roofing or iron-sheet roofing. Data collected for 20 consecutive days in February 20011.

| Mean (SD) indoor temperature (°C) | | Mean (SD) relative indoor humidity (%) | | |
|-----------------------------------|--------------------------|--|------------------------|------------------------|
| | Local grass-thatched hut | Ifakara experimental hut | Local iron-roofed huts | Local iron-roofed huts |
| Day | 26.5 (±1.3) | 27.5 (±2.3) | 29.2 (±2.4) | 83.2 (±8.4) |
| Night | 26.1 (±1.0) | 25.1 (±1.7) | 26.8 (±1.2) | 89.4 (±4.4) |

Table 2: Mean and standard deviations (SD) of daily temperatures and humidity inside and outside Ifakara experimental huts. Data collected between May and October 2010

| Temperatures (°C) | | Relative Humidity (%) | | | |
|-------------------|------|-----------------------|------|----------|-------|
| Indoors | | Indoors | | Outdoors | |
| | SD | Mean | SD | Mean | SD |
| Day | 3.96 | 28.13 | 5.33 | 66.49 | 18.42 |
| Night | 2.50 | 20.66 | 2.16 | 67.55 | 10.73 |

Table 3: Mean daily wind speeds and cumulative rainfall outside Ifakara experimental huts between May and October 2010

| Wind speeds (Km/h) | | Cumulative rainfall (mm) | |
|--------------------|------|--------------------------|------|
| | SD | Mean | SD |
| Day | 3.31 | 758.91 | 5.66 |
| Night | 2.44 | 758.81 | 5.69 |

Molecular analysis of mosquitoes

PCR analysis of the *An. gambiae* s.l samples from the field studies showed that among the 1524 successful individual mosquito DNA amplifications, 96.7% were *An. arabiensis* (n = 1474) and 3.3% were *An. gambiae* s.s (n = 50). No molecular analysis was conducted for the other malaria vector, *An. funestus* complex mosquitoes, a few of which were also caught during these studies.

Entry and exit behaviour of local malaria vectors in the study area

It was determined that the main malaria vector in the study area, *An. arabiensis* prefers to enter houses via eaves but to exit via windows, and that these mosquitoes exit houses mainly in the early morning hours between 3.00am and 7.00am. The number of mosquitoes entering huts at different times was generally equal throughout the night except for two small peaks, the first between 10pm and midnight and the second slightly more pronounced peak between 3am and 5am.

Effects of baffles on exiting mosquito catches

Addition of the inward facing netting barriers (baffles) to the eave spaces of the experimental huts ensured that greater proportions of mosquitoes that entered the huts were retained and captured in the exit traps (Table 4). The trap catches were higher whenever baffles were used in the experimental huts relative to when no baffles were used. When data were aggregated by hut and day, the presence of baffles increased the number of *An. arabiensis* collected from a geometric mean (95% CI) of 64.68 (45.35-92.24) to 96.27 (69.79-132.81). This increase was statistically significant for *An. arabiensis* Relative Rate (RR) 1.44 (1.17-1.77), $z=3.46$, $p=0.001$, and total

mosquitoes collected RR (95% C.I.) 1.38 (1.10-1.73), $z=2.82$, $p=0.005$. When data for each trap type was analysed the use of baffles increased the likelihood of *An. arabiensis* being trapped in a window exit trap RR (95% C.I.) = 1.57 (1.03 – 2.37), $z=2.13$, $p=0.033$; and more than doubled the likelihood of *An. arabiensis* being trapped in an eave exit trap RR (95% C.I.) = 2.90 (1.89 – 4.48), $z=4.84$, $p<0.0001$. When used with baffles, the number of mosquitoes recovered from window traps is not significantly different from CDC light traps indicating good sampling efficiency. The data (Table 4) also confirms that, even though *An. arabiensis* prefers to enter huts via eaves spaces rather than window spaces, these same mosquitoes tend to exit huts mainly via windows as opposed to eave spaces. The catches in light traps with baffles were also higher, indicating that the baffles did not inhibit mosquito hut entry.

Effects of para methane 3, 8, diol (PMD) on the number of mosquitoes entering the experimental huts

Table 5 shows a summary of mosquito catches in huts sprayed with PMD and huts left as controls over the 6 experimental nights. In huts fitted with entry traps, there was a 49% reduction in median number of *An. arabiensis* mosquitoes caught in PMD sprayed huts compared to control huts. Median catches of *Culex* mosquitoes were reduced by 43% and *Mansonia* species by 20% (Table 5). When this data was subjected to generalized linear models, we observed no significant effects of PMD spraying on catches of any of these species even though the Relative Rates of mosquito catches were conspicuously lower than 1. The RR (95% CI) of *An. arabiensis* catches in PMD sprayed huts compared to control huts was 0.48 (0.21 - 1.08), $z = 1.78$, $df=1$, $P = 0.075$. Relative Rate for *Culex* mosquitoes was 0.80 (0.34 – 1.89), $z = 0.51$, $df=1$, $P = 0.610$ and that for *Mansonia* species was 0.53 (0.22–1.23),

$z = 1.44$, $df = 1$, $P = 0.151$). We observed no significant effect of huts themselves on number of mosquitoes caught. Interestingly, we observed no reduction due to PMD treatment in any of the huts that were fitted with exit traps (Table 4). This was true for *An. arabiensis* ($RR = 1.08$ (0.49-2.42), $z = 0.19$, $df = 1$, $P = 0.845$), for *Culex* species ($RR = 0.82$ (0.34-1.89), $z = 0.46$, $df = 1$, $P = 0.643$) and for *Mansonia* species $RR = 1.19$ (0.52-2.75) $z = 0.41$, $df = 1$, $P = 0.678$). However the overall exit trap catches in PMD huts was higher than in control huts, suggesting that the presence of PMD was irritating and forcing excess mosquitoes out of the treated huts. This irritant effect accounted for 15.5% excess exit of *An. arabiensis* mosquitoes, even though this was not a statistically significant increase relative to the control.

Table 4: Geometric Mean (GM) catches and the 95% confidence intervals (CI) of *Anopheles arabiensis* mosquitoes in the Ifakara experimental huts whenever baffles were used compared to when no baffles were used. The Relative Rate (RR) and 95% confidence intervals (CI) of the mosquito counts in traps when baffles are used relative to when they were not used were calculated from Generalized Linear Models.

| Trap type | <i>Anopheles arabiensis</i> | | | | | | Total mosquitoes | | | | | |
|-------------------|-----------------------------|----|---------|--------------|----|---------|------------------|----|---------|-----------------|----|---------|
| | Without baffles | | | With baffles | | | Relative Rate | | | Without baffles | | |
| | GM | CI | P | GM | CI | P | RR | CI | P | GM | CI | P |
| CDC | 45.77 | | | 69.92 | | | 1.07 | | | 98.25 | | |
| | 74.48 | - | 0.009 | 96.69 | - | 0.009 | 1.29 | - | N.S. | 152.66 | - | N.S. |
| Window exit traps | 121.18 | | | 133.69 | | | 1.57 | | | 237.19 | | |
| | 16.53 | | | 31.74 | | | 1.03 | | | 45.06 | | |
| Eave exit traps | 35.28 | - | 0.033 | 61.00 | - | 0.033 | 1.57 | - | 0.009 | 72.76 | - | 0.009 |
| | 75.32 | | | 117.16 | | | 2.37 | | | 117.47 | | |
| Eave exit traps | 3.42 | | | 8.93 | | | 1.89 | | | 4.96 | | |
| | 5.89 | - | <0.0001 | 15.39 | - | <0.0001 | 2.90 | - | <0.0001 | 8.36 | - | <0.0001 |
| Eave exit traps | 10.04 | | | 26.54 | | | 4.48 | | | 14.11 | | |
| | | | | | | | | | | 35.39 | | |

Table 5: Median number of mosquitoes of different species caught in Ifakara experimental huts that were either sprayed with PMD or left unsprayed. The huts were fitted with either entry traps or exit traps. Values in parenthesis represent inter-quartile ranges. Percentage reduction of mosquito catches due to PMD, was calculated based on the median mosquito catches is also shown

| Traps fitted | | <i>Anopheles arabiensis</i> | | | <i>Culex species</i> | | | <i>Mansonia species</i> | | |
|-----------------------|--|-----------------------------|------------------------|------|----------------------|------|--------------------|-------------------------|--------------|-----|
| | | Treatment | Median (IQR) | Sum | Median (IQR) | Sum | Median (IQR) | Sum | Median (IQR) | Sum |
| Huts with entry traps | | Control Huts | 22.5 (7.5-71.3) | 470 | 3.5 (1.0-10.8) | 90 | 5.0 (3.0-14.5) | 100 | | |
| | | PMD sprayed Huts | 11.5 (4.3-31.0) | 224 | 2.0 (0.0-8.8) | 72 | 4.0 (3.0-6.0) | 53 | | |
| | | % Reduction | | 48.9 | | 42.9 | | 20.0 | | |
| Huts with exit traps | | Control Huts | 152.0 (109.5-212.0) | 1889 | 10.5 (6.8-16.5) | 174 | 10.0 (4.3-17.5) | 124 | | |
| | | PMD sprayed Huts | 175.5 (129.5-218.5) | 2046 | 10.0 (7.3-14.0) | 143 | 11.0 (9.0-16.0) | 148 | | |
| | | % Reduction | | 0 | | 5 | | 0 | | |

Comparison of the number of mosquitoes caught while entering or exiting experimental huts fitted with entry traps or exit traps alone versus experimental huts fitted with both entry traps and exit traps

Trap arrangement (i.e. whether the huts are fitted with entry traps only or with a mixture of entry and exit traps) affected the number of mosquitoes caught, even though in some cases, these differences were only marginally significant. The number of *An. arabiensis* caught exiting the huts (i.e. exit trap catches) was higher in huts fitted with only exit traps than in huts fitted with a mixture of exit and entry traps (RR = 1.24 (0.98-1.57), $z = 1.78$, $df = 1$, $P = 0.076$). Similarly, when mosquitoes were caught while entering huts (i.e. in entry traps), *An. arabiensis* catches were higher when the huts had only entry traps compared to when the huts had a mixture of entry and exit traps (RR = 1.65 (1.12-2.45), $z = 2.50$, $df = 1$, $P = 0.012$). We observed similar differences but with more pronounced statistical significance levels for *Culex* and *Mansonia* species mosquitoes. Specifically, in exit traps, the Relative Rate of *Culex* catches in huts fitted with only exit traps compared to huts fitted with both exit and entry traps was 1.50 (1.20-1.88), $z=3.57$, $P<0.0001$ and in entry traps the RR was 1.84 (0.95-3.54), $z= 1.81$, $P=0.071$. In the same order, the RR for *Mansonia* species in exit traps were 1.80 (1.16-2.80), $z= 2.61$, $P=0.009$ and 1.45 (0.88-2.41), $z=1.67$, $P=0.149$ in entry traps.

Overall, the entry traps caught only about one eighth of all mosquitoes of all species that were collected in exit traps. In huts having a mixture of entry and exit traps, 90.4% of the *An. arabiensis* were caught in the exit traps, 8.4% in the entry traps and only 1.2% inside the huts, having failed to exit. On the other hand, in huts with only exit traps, 98.4% were caught in the exit traps and 1.6% inside the huts having failed to exit. Table 6 shows a summary of mosquito catches (median, inter-

quartile ranges and sum of mosquitoes collected when huts were fitted with either one type of trap or with a mixture of entry traps (50%) and exit traps (50%).

Comparison of the number of mosquitoes entering different experimental huts

Summaries of catches for the different mosquito species in the 9 huts tested here are included in Table 7. Differences in mosquito catches between the huts was analysed using generalised linear models (GLM) based on totals of mosquitoes caught per night per hut, fitted in a negative binomial distribution model with a log link function. Using either the first hut (hut 1) or the last hut (hut 9) as reference, we observed that *An. arabiensis* catches in all the other huts were always significantly different from these huts ($z = 6.00$, $df = 8$, $P < 0.001$). This was also true for *Mansonia* species ($z = 6.07$, $df = 8$, $P < 0.001$), but not for the *Culex* species ($z = 3.62$, $df=8$, $P = 0.108$) collected in the huts.

To identify the actual huts contributing to these differences, we conducted a univariate GLM on log transformed *An. arabiensis* catches, with *post hoc* analysis using Tukey's Honestly Significant Difference test. While this test confirmed an overall significant difference between catches in individual huts ($F=2.859$, $df=8$, $P=0.005$), two important findings emerged. First, hut 1 and hut 9 were the most different from the others. Second, the differences were significant only when we directly compared hut 1 versus hut 2 ($P=0.013$) or hut 1 versus hut 9 ($P=0.004$), but not any other pair of huts ($P>0.05$). When we eliminated catches from huts 1 and hut 9 and redid the analysis on the rest of the data, there were no significant differences between huts for *An. arabiensis* ($z=3.13$, $df=6$, $P=0.133$) and *Culex* species ($z=3.02$, $df=6$, $P=0.165$) but not *Mansonia* species ($z=5.64$, $df=6$, $P<0.001$)

Table 6: Median number of mosquitoes caught entering or exiting huts fitted with different trap arrangements namely: 1) entry traps only, 2) exit traps only or 3) a mixture of entry traps and exit traps. These medians were calculated per hut per night. Values in parenthesis represent inter-quartile ranges

| | Experimental huts fitted with entry traps only | | Experimental huts fitted with exit traps only | | Experimental huts fitted with a mixture of entry traps (50%) and exit traps (50%) | |
|---------------------------|--|-------------------------------------|---|------------------------|---|------------|
| | Median (IQR) | Sum | Median (IQR) | Sum | Median (IQR) | Sum |
| Exit trap catches | | | | | | |
| | <i>An. arabiensis</i> | - | - | 211.0 (142.5-323.0) | 176.5 (91.3-267.0) | 10,263 |
| | <i>Culex</i> species | - | - | 8.0 (5.0-11.0) | 5.0 (3.0-8.3) | 309 |
| | <i>Mansonia</i> species | - | - | 10.5 (6.0 - 19.0) | 7.5 (4.0-12.3) | 528 |
| Entry trap catches | | | | | | |
| | <i>An. arabiensis</i> | Median (IQR) 25.0 (17.0-34.3) | Sum 1,575 | Median (IQR) - | Median (IQR) 13.0 (6.0-26.5) | Sum 952 |
| | <i>Culex</i> species | 2.5 (1.0-4.3) | 189 | - | 1.0 (0.0- 2.3) | 103 |
| | <i>Mansonia</i> species | 5.0 (3.0-9.0) | 363 | - | 3.0 (1.0-6.3) | 250 |

Table 7: Median number of mosquitoes collected per night in individual experimental huts. Values in parentheses represent inter-quartile ranges.
 For *Anopheles arabiensis* letters that differ denote statistical significance

| | <i>Anopheles arabiensis</i> | | <i>Culex species</i> | | <i>Mansonia species</i> | |
|--------------|------------------------------------|------|----------------------|-----|-------------------------|-----|
| | Median (IQR) | SUM | Median (IQR) | SUM | Median (IQR) | SUM |
| Hut 1 | 44.0 ^a (23.3-75.5) | 922 | 4.0 (1.0-5.3) | 75 | 7.0 (5.0-12.3) | 166 |
| Hut 2 | 228.5 ^b (44.0-409.0) | 4488 | 3.5 (2.0-8.3) | 99 | 18.0 (8.8-43.3) | 528 |
| Hut 3 | 168.5 ^b (25.0-223.0) | 2514 | 4.5 (2.0-6.3) | 87 | 6.0 (4.0-9.3) | 126 |
| Hut 4 | 76.0 ^b (31.0-165.8) | 1810 | 5.0 (2.0-10.0) | 109 | 8.5 (5.0-13.0) | 218 |
| Hut 5 | 152.5 ^b (32.5-213.8) | 2613 | 10.0 (2.8-15.0) | 183 | 28.0 (9.0-33.5) | 482 |
| Hut 6 | 133.5 ^b (34.3-186.3) | 2227 | 7.5 (6.0-10.3) | 184 | 12.0 (8.8-17.3) | 248 |
| Hut 7 | 144.0 ^b (44.5-256.8) | 2971 | 5.5 (2.5-7.3) | 93 | 7.0 (4.8-12.5) | 156 |
| Hut 8 | 195.5 ^b (29.8-338.5) | 3602 | 5.0 (3.8-10.0) | 126 | 10.0 (1.8-13.0) | 158 |
| Hut 9 | 313.0 ^b (54.8-435.8) | 4805 | 8.5 (4.8-12.3) | 149 | 7.5 (3.8-12.5) | 145 |

Discussion

The design of Ifakara experimental huts has been accomplished by combining advantageous design elements from several experimental huts previously used in mosquito studies [24]. Moreover, this design is an attempt to improve upon limitations identified in many of those previous huts. The final design of these new experimental huts has incorporated: 1) improvements on actual physical structure to make them more representative of local houses, 2) mosquito trapping methods that maximise mosquito entry and recovery as well as representative assessment of mosquito exposure to insecticides, 3) improved geographical positioning of the huts within the study area to maximise mosquito numbers while minimising disturbance to local residents; and 4) a suite of customised experimental practices employed when working with these experimental huts.

Some of the practical advantages of these huts are: 1) they are made in kit-format and can therefore be easily disassembled , transported between different sites and re-assembled onsite, 2) the possibility to replace the mud panels and the ceiling, whenever a new insecticidal application is to be done so that all insecticides may be disposed of safely, 3) their similarity in style and size to local houses commonly used in the study area, which effectively improves their representativeness and 4) the fact that these huts, despite being fitted with traps all-round, still have adequate spaces for mosquitoes to enter. The huts can accommodate two human volunteers, who can both act as baits to lure in mosquitoes but also as mosquito collectors thus improving attraction to mosquitoes and maximising recovery of mosquitoes. This is clearly reflected in the high numbers of mosquitoes including the malaria vector *An. arabiensis* recovered from huts on a regular basis during our studies.

In our preliminary behavioural assays, for which results have been presented here, we observed clearly that *An. arabiensis* prefers to enter huts through eave spaces, but that these mosquitoes exit mainly through windows. We expected however, that if chemical-based interventions with irritant effects are used inside the huts, the mosquitoes may be forced to exit the huts via any available and nearest exits including the eaves [34, 58], thus disrupting the natural exit pattern. As Ifakara experimental huts with baffles collect similar numbers of mosquitoes in exit traps as CDC LT, these specific challenges have been overcome in the design. Results of these experiments evidently show that the baffles do indeed boost exit trap catches, by retaining mosquitoes, which would otherwise exit unmonitored. It is also important to note from these results that presence of the baffles did not in anyway alter the entry pattern or the number of mosquitoes that entered the experimental huts.

Clearly, when evaluating household insecticide applications, these baffles become an even more important component of experimental huts, since they also guard against possible overestimation of percentage mortality due to candidate interventions. It is known that irritated mosquitoes tend to exit experimental huts through any opening including eave spaces [34], meaning that where there are no baffles, the sum of remaining mosquitoes, which is normally used as the denominator when calculating percentage mortality [19], will obviously be less than total number of mosquitoes that actually entered the huts. A good example of this can be found in early reports of work done by Dr. Alec Smith in northern Tanzania [35]. In one study investigating effects of an insecticide, dichlorvos, on mosquitoes visiting experimental huts, he observed that whenever mosquitoes leaving huts through the eave spaces were considered in his equations, the calculated mortality was always lower than whenever eave egress fraction was ignored [35]. Even with purely toxic

and non-irritant insecticides, only the live mosquitoes would have a chance to escape, thus leaving mostly knocked down or dead ones inside the huts, a situation which can lead to an overestimation of proportions mosquitoes that die inside the huts, as a direct result of the insecticidal intervention being evaluated [34-36]. Therefore, we strongly recommend the use of baffles when evaluating insecticides in experimental huts.

In addition to the baffles, mosquito collection from all four sides of the huts on any given night, has some advantages over collection from only two opposite sides, which has been a common practice in previous studies involving veranda-type experimental huts [19, 34, 59-61]. This way, biases that may result from differences in directions of wind and light are minimised. Moreover, researchers also eliminate potential statistical problems associated with the previous practice of doubling the number of mosquitoes caught, so as to obtain the sum of mosquitoes that could have visited the huts if the collections were conducted on all sides of the huts [59-61]. Indeed, we have directly observed in our study area that this practice could be invalid, since the numbers of mosquitoes entering huts through any two opposite sides are never equal and in experiments where baffles were not used loss of mosquitoes is also not be equal on any two opposite sides, or exactly half of total entry.

Similarly, sampling mosquitoes on all sides, ensures that the open areas available for mosquitoes to enter the experimental huts is greater than seen among other hut designs, especially those previously used in west Africa, which allow mosquitoes to enter only via very small, 1cm wide, window slits on three sides of each hut [62-64]. Again, we have demonstrated in our study sites in south-eastern Tanzania, that the malaria vector *An. arabiensis* prefers entering houses via eave spaces rather than through windows [40], but also that more mosquitoes enter huts if a

greater area of the eave space is left unobstructed. This may suggest that the common west African experimental hut design such as the ones used in Benin [62] may not necessarily be as suitable for studying this East African vector population, as they have been for west African mosquito populations.

Another factor that has been addressed by the design described in this paper is prolonged mosquito retention within exit traps. It was observed during some early hut studies conducted in the 1960s that whenever mosquitoes were confined for long periods inside exit traps attached to insecticide treated experimental huts, there was excess mortality of mosquitoes in these traps, presumably due to concentrated fumes of the insecticides or accumulated insecticide dust deposits inside these traps [65, 66]. Despite these early observations, a common practice in current experimental hut studies is that mosquitoes remain held for long hours inside the exit traps or in verandas, and are removed only in the morning [19, 24], potentially increasing the probability of death as a result of this extended exposure to insecticide fumes.

One solution earlier proposed by Smith and Webley in 1963, was that insecticide-proof materials such as transparent polythene sheeting could be used to cover the side of window traps facing inside the experimental hut [66]. As described earlier, the traps used on the Ifakara experimental huts are all made entirely of netting, and instead the possibility of excessive mortality is minimised by regularly emptying the traps several times each night, so that the mosquitoes do not remain confined inside the traps and in close proximity to any insecticide fumes that could be emanating from the houses. This is usually done every 1-4 hours depending on research questions and associated logistical constraints. Once removed from the exit traps the mosquitoes are immediately transferred to a field insectary, 100m away from the experimental huts, where they are maintained on 10% aqueous solution of glucose

and monitored, usually for 24 hours. Other than being merely an attempt to minimize excessive mortality, this practice of multiple collections per night also more representatively matches what free-flying wild mosquitoes do around houses in real life; given that any mosquitoes found in the exit traps, are those that would otherwise have escaped completely from the huts. Moreover, such multiple collections now make it possible to identify and quantify irritant effects of insecticides which induce mosquitoes to exit huts earlier than usual [1, 58]. In fact, in previous experimental hut evaluations of insecticidal interventions in Africa, the closest estimates of irritancy were those based on overall differences between proportions of mosquito catches that were found in the exit traps in treatment versus control huts, and that in most cases, no attempts were actually made to assess whether insecticides induced earlier exit than normal [67]. This modification to allow multiple mosquito collections each night is therefore an essential improvement specifically in relation to huts previously used within Africa, which did not consider this aspect.

The third important practice conducted as part of the assay is blocking of some hut windows during the day. This is normally done in order to minimise potential effects of wind, i.e. the likelihood that any insecticides sprayed inside the experimental huts can be gradually eroded and blown around by wind, leading to rapid decay of the desired efficacies of candidate residual insecticides, while at the same time accumulating the eroded insecticide particles inside exit traps attached to the huts. Though the Ifakara experimental huts have 4 windows all of which are fitted with interception traps, 3 of the windows are usually covered during the day using tightly fitting pieces of canvas. These canvas covers are placed from the inside of the huts, effectively blocking the front part of the window traps during the day. They are however removed every evening so that all the 4 window traps can be used to collect

mosquitoes during the night. Again, other than minimising effects of wind, our direct observations confirm that this particular practice correctly matches what normally happens in most local houses in southern Tanzania, where at least some of the windows are kept partially covered with curtains or wooden shutters during the day, or the windows remain fully closed.

Lastly, we initially observed that every evening just before our experiments began there were already a number of mosquitoes inside the huts. Since no volunteers stayed inside the huts during the day, and because most of these early mosquitoes were unfed, it is possible that either the mosquitoes entered the huts to rest [68] or they were lured by residual odours left behind by volunteers from the previous nights, and entered the huts anticipating blood meals [45, 68]. Experimental evaluations should therefore involve not only night-time collections, but also daytime collections where possible. Though such daytime collections are nowadays hardly conducted in experimental hut studies [19], early hut practitioners paid great attention to mosquitoes resting inside huts during the day [26]. In Ifakara experimental huts, collections targeting mosquitoes that may have entered huts during the day are done every evening between 1800Hrs and 1900Hrs, just before volunteer sleepers enter the huts to begin the night time catches. When testing interventions such as ITNs, which can be rotated daily or weekly between huts, the time when these nets are put into designated huts, i.e. whether this is done in the mornings or in the evenings, must be carefully considered so that these daytime effects are attributed to the right net type. Here also, inclusion of day time catches more representatively captures the 'round-the-clock' interactions between mosquitoes and insecticidal interventions, when used inside local homes, than the current practice of monitoring only those mosquitoes visiting experimental huts at night [19].

Experiments conducted using a mosquito repellent PMD [53, 54], verified the suitability of the Ifakara experimental hut design in studies to assess effects of various insecticidal compounds on malaria mosquitoes. By corroborating the reduction in number of mosquitoes caught inside PMD sprayed experimental huts relative to unsprayed huts and by being able to monitor all mosquitoes coming and leaving the huts, the tests provided a useful opportunity for identifying limitations in our procedures and also the necessary adjustments prior to subsequent studies using these huts. For example, we proved that emptying the traps every four hours is logistically possible on a routine basis, and as such this procedure was adopted for subsequent experiments.

Other than these observations, this particular experiment itself demonstrated the necessary training required for both the field technicians and the participating volunteers, on a wide range of entomological procedures involved in experimental hut evaluation of insecticidal interventions. We must also point out at this stage that even though these preliminary tests were carried out using just PMD (selected because it is a botanical with no long-term residual effects [53, 54]), it is logical to infer from the process and also from the results that indeed, these huts can be used to evaluate different insecticidal applications including LLINs and IRS, which may not have exactly the same mode of action as PMD. For example certain insecticides commonly used in ITNs e.g. permethrin [69-71] and also insecticides used for IRS e.g. the pyrethroid, lambda cyhalothrin [62, 72-74] and the organochloride, DDT [59, 75-78], are known to be not only toxic to mosquitoes, but also repellent and can be evaluated using these experimental huts. Given the specific reasons for using PMD in this study, we did not consider it essential to incorporate any assays to determine residual content

of the compound on treated hut walls, and therefore we are unable to determine how its effects on mosquitoes would change over time.

One particularly crucial observation during this experiment was that while reduction in mosquito catches due to PMD could be readily detected in huts fitted with entry traps, this was not the case in huts fitted with exit traps, in which PMD related reduction was 0% for *An. gambiae s.l* and *Mansonia* mosquitoes, and only 5% for *Culex* mosquitoes. It certainly raises concern as to whether exit traps alone could be adequate to evaluate insecticides which also have these deterrent properties. However, because we also observed a minor increase in *An. arabiensis* catches inside exit traps fitted on PMD sprayed huts, relative to traps fitted on control huts, one would argue that exit traps are more suitable for measuring irritant effects of treatments upon mosquitoes that are already inside the huts, while entry traps are better when assessing how different treatments deter mosquitoes from entering the huts in the first place. The PMD repellence therefore can only be clearly observed if one considers entry trap catches, which however are evidently are only a small fraction compared to exit trap catches as the two methods do not have the same sampling efficacy. What is undoubtedly clear from this preliminary evaluation is that there is a significant difference in trapping efficiencies between exit traps and entry traps.

Whereas combination of entry and exit traps provides an opportunity to study both entry behaviour and exit behaviour of mosquitoes concurrently, thus avoiding nightly variations in mosquito catches, our tests showed that using all exit traps in each hut collects more mosquitoes than when a combination of entry and exit traps are used. Moreover, the number of mosquitoes entering the huts could be grossly underestimated if only the entry traps are used; since these traps capture only about

13% of all mosquitoes that actually enter the huts. These experiments also showed that most of the mosquitoes were caught in exit traps, even though there was no insecticidal application used in the huts. These findings suggest that in the absence of any intervention, exit traps are more efficient than entry traps, therefore rather than combining the trap types, it is better to use only exit traps, interspersed with spaces fitted with baffles. Given that variation (as depicted by inter-quartile ranges) were not different for the different trap arrangements, the assertion that it is better to use exit traps can be based only on improved catches, but not on the fact that such a practice would reduce data variability. Moreover, that assertion may not be interpreted to mean that exit traps are always better than entry traps in experimental hut studies. On the contrary, it should be noted that the type of interception trap to fit must be guided by whatever research questions are being addressed. Moreover, it should also be noted that that even though exit traps performed multiple times better than entry traps in this study, both trap types are actually physically the same, except that one type is fitted facing the inside of the huts (entry traps), while the other is fitted while facing the outside (exit traps).

Entry traps for example, may have lower trapping efficiencies than exit traps, but as depicted by our PMD test results, these traps are clearly better for assessing repellent effects of interventions, than exit traps. Exit traps on the other hand, if used together with baffles would be better for examining toxicity and irritant effects of interventions. Similarly, where the interest is to also determine the actual time when mosquitoes enter houses, then entry traps emptied frequently, say hourly would be more useful than exit traps, which do not account for mosquitoes dead or knocked-down within the huts. Nevertheless, where exit traps are used, it is necessary that additional collections are done indoors using mouth aspirators, to retrieve mosquitoes

that fail to exit huts. All these are essential considerations when assessing house-hold level protective efficacies of interventions. Therefore, users of these experimental huts must ensure that the trap arrangement used suits the intended purposes

In experiments where mosquito catches were compared between the different huts, there was variation between huts in mosquito density. These differences may be related to either the positions of these huts [14] or to the differences in attractiveness of the human volunteer pairs who slept in the huts [4, 5]. One limitation of this experiment was that due to the need for logistical simplicity and statistical replication the human volunteers did not rotate between the huts. As such, hut plus the volunteers assigned to that hut were treated as a single source of bias and it is therefore difficult to identify the proportion of this effect that was actually caused by the positional differences between huts. Nevertheless, the advance knowledge of these differences was important in informing design of subsequent experiments, in which candidate insecticidal interventions and controls that could not be rotated (IRS) were now randomly assigned several huts to increase replication and where possible, treatments (LLINs) rotated between huts at different positions, while retaining the volunteers in their respective huts.

One of the primary goals of the previous hut developers was to create huts that resembled local human houses, and the Ifakara experimental huts are therefore not the first huts to attempt matching designs of local houses in study areas. Nevertheless, we present these huts as an improvement relative to the existing hut designs, which arguably, did not fully achieve the goal of matching local houses. For example, the East Africa veranda trap huts are very small and would not necessarily have similar airflow as local houses [34, 60]. Similarly, the West African huts such as those used in Benin [62], allow mosquitoes to enter huts via very small slits on the sides, thus

restricting the natural entry pattern and adjusting the airflow in the huts. Also the, way mosquitoes are collected in many of these existing huts, usually by retaining them in close proximity to the huts until morning, may not necessarily represent the natural behaviours of mosquitoes, especially where users are protected with nets. For instance, our own observation of *An. arabiensis* in this study site, suggests that when these mosquitoes enter huts where volunteers are protected with nets, they do not necessarily spend a long time inside those huts, but that instead, they readily exit the huts, presumably to continue host seeking elsewhere. Retaining the mosquitoes till morning in a veranda trap, would therefore possibly lead to longer exposure to whatever interventions are in applied in the huts. In light of the above examples, we recognize that though the Ifakara experimental huts may not in themselves be the perfect match to local houses they constitute an improvement towards this goal, especially since the existing east and west African hut designs have not been modified for many decades.

Despite these improved characteristics of the Ifakara experimental huts, we cannot at this stage propose this design as a replacement of any existing hut designs. We recognise that perhaps the most important issue in that regard is the need to directly compare different hut designs currently being used in Africa and assess their relative efficacies for assessing effects of indoor interventions on mosquitoes. Nevertheless, one must also consider the value of data that such comparisons would produce, and how generalizable the conclusions of any one study location would be to different locations, given the diversity of local house designs in Africa, but also the differences in house-entry and feeding behaviours of mosquitoes in different places. Moreover, since experimental huts that are currently being used have different functional mechanisms and sizes, and because it may not be possible to fit them with

exactly the same types of interception traps, another challenge to direct comparison of hut types would be how to decide on output variable to measure, and how exactly that variable should be measured.

Therefore, even though this manuscript is limited to the description and preliminary testing of the Ifakara experimental huts as an alternative option when evaluating indoor interventions against East African mosquito populations, we strongly recommend that prospective users should independently assess the utility of the huts in their respective localities before using them. In addition, the entomological procedures described here provide a framework that may also be modified to more accurately match intended research purposes and to better evaluate effects candidate interventions being tested.

Conclusion

The Ifakara experimental huts provide a more realistic system that can be used to study the natural behaviour of wild free-flying populations of disease-transmitting mosquitoes, including the increasingly dominant African malaria vector, *An. arabiensis*, and to evaluate efficacy of various indoor vector control technologies. Their efficacy is enhanced by the improved design relative to previous hut designs, specifically the fact that mosquito entry is maximised to improve the power of evaluations. The huts use both eave and window traps thus making the design suitable for studying a wide range of mosquito entry and exit behaviours and the nature of traps fitted onto the traps, the use of eave baffles to control mosquito exit improves data reliability. The huts are designed to be an assay with the use of replaceable wall panels and ceilings, and the kit format of the huts, but also by the specific entomological practices used to sample mosquitoes in these huts.

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Chapter IV

Comparative evaluation of combinations of long lasting insecticidal nets and indoor residual spraying, relative to the use of either method alone, for malaria vector control in an area dominated by *Anopheles arabiensis**

Abstract

Background: Malaria vector control in sub-Saharan Africa is currently practised using long lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS). In several highly endemic regions both methods are used within the same household although there is limited direct empirical evidence to demonstrate advantages of employing both methods simultaneously. The purpose of this study was to determine if there is any such advantage relative to using either method alone.

Methods: Comparative evaluations were conducted in experimental huts fitted with LLINs alone, IRS alone, or combinations of LLINs and IRS, in an area where *Anopheles arabiensis* is the predominant malaria vector. Indicators of protection included: 1) number of mosquitoes entering huts, 2) proportion and total number of mosquitoes killed after exposure to each treatment, 3) time when mosquitoes exited the huts, 4) proportions of mosquitoes prevented from feeding upon volunteers sleeping inside the huts, and 5) proportions caught exiting the huts. Three intact LLIN types (Olyset®, PermaNet 2® and Icon Life® nets) and three IRS treatments, actellic (organophosphate), DDT (organochloride) and lambda cyhalothrin (synthetic pyrethroid), all applied at WHO recommended doses, were assessed singly or in combinations, relative to non-insecticidal nets used alone. The study was conducted in two spray rounds, I and II.

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Findings: All net types provided nearly full protection from mosquito bites (> 99% feeding inhibition) regardless of whether they were used in combination with any IRS or not. Addition of LLINs into huts with IRS provided additional protection through feeding inhibition, with PermaNet® and Icon life® nets also increasing the proportions of malaria mosquitoes killed. Deterrence of mosquitoes was not observed with LLINs, except a 30% reduction of *An. arabiensis* catches in huts with PermaNet® nets during spray round 1. Addition of IRS using DDT deterred more mosquitoes from huts already having LLINs, but did not increase proportional mortality. In contrast, IRS with actellic significantly increased proportional mortality relative to LLINs alone, but did not induce any deterrence. Lambda cyhalothrin increased mortality to a minimal extent, and had no deterrence. More than 95% of mosquitoes were collected in exit traps rather than inside huts.

Conclusions: 1) there are only minimal additional protective benefits achievable from adding IRS in houses where people already correctly and consistently use LLINs, 2) intact untreated nets, by preventing mosquito bites, can effectively complement IRS, where LLINs are not readily available, therefore in places where IRS is used, efforts should be made to also provide at least untreated bed nets, 3) LLINs/IRS combinations would be most protective if the IRS was based on highly toxic and less irritant non-pyrethroids such as actellic: combinations which would also mitigate against insecticide resistance, and 4) where resources are limited, the focus of malaria control should be to ensure that all people at risk use LLINs consistently, instead of trying to implement both LLINs and IRS.

Background

Much of the recent reduction in malaria has been attributed primarily to the two most common malaria vector control methods, namely, insecticide treated nets (ITNs), and indoor house spraying with residual insecticides (IRS) [1-8]. These methods are currently supported by an exemplary level of public and political goodwill, and are complimented by other recent advances such as prompt and accurate diagnosis [9-11], treatment with artemisinin based medicines [3, 5, 12-14], and intermittent preventive treatment (IPT) [15, 16], all of which have also significantly contributed to the gains accrued.

Though long-lasting insecticidal nets (LLINs) are designed as stand-alone vector control tools, there are several instances where they are combined with IRS in the same houses, often with the aim of achieving greater health benefits. In an earlier review article (Chapter 2) [17], we examined potential advantages of combining LLINs with IRS, and outlined measures that could ensure maximum efficacy of such combinations. We also noted that other than a small amount of indirect field evidence [18-21], and an assortment of theoretical simulations [22-24] suggesting added advantages of the combinations relative to either LLINs or IRS alone, there had not been any studies that explicitly determined whether combining LLIN with IRS in the same households would have synergistic or redundant effects [17]. Since that review, at least one study conducted in Benin has now showed that combinations of deltamethrin-based LLINs with chlorfenapyr, a pyrethroid insecticide, have potential to not only provide additional protection relative to the components singly, but also that such combinations can be effective against insecticide resistant vector populations [21].

There are several theoretical justifications for combining LLINs with IRS, and consequently a need to optimise this strategy. We have previously suggested that: 1) any complementary IRS insecticides should have different modes of action from the pyrethroid-based LLINs, 2) the overall community-level epidemiological outcomes of any LLIN/IRS co-applications would be modulated by factors such as the extent of intervention coverage in the communities, baseline epidemiological conditions and the behaviour of local malaria vectors [17], and 3) that a series of studies should be conducted to generate direct evidence for or against these combinations.

The purpose of this current study was therefore to contribute essential empirical evidence on protective efficacy of LLIN/IRS combinations in a malaria endemic area. Through comparative evaluation, we observed various indicators of protection inside experimental huts [25], where both LLINs and IRS were used, and compared these with similar observations in huts where either LLINs alone, IRS alone or non-insecticidal nets were used. Given that WHO-approved LLINs have different active ingredients, and because there are several classes of insecticides approved for IRS [26], this study involved multiple combinations of net types and IRS insecticides.

Materials and methods

Study area

The study was conducted in Lupiro village (8.385°S and 36.670°E) in Ulanga District, south eastern Tanzania. The village lies 300m above sea level, and is 26 km south of Ifakara town, where Ifakara Health Institute (IHI) is located. It borders many small contiguous and perennially swampy rice fields to the northern and eastern sides. The annual rainfall is 1200-1800mm, while temperatures range between 20°C and 32.6°C. Composition of malaria vector populations (which previously included a mixture of

Anopheles gambiae and *Anopheles funestus* complexes, the former consisting predominantly *An. gambiae sensu stricto*) has shifted dramatically in recent years, most likely because of high ITN coverage [27]. Today, the most common vector is *Anopheles arabiensis*, constituting > 95% of the *An. gambiae* complex species [28, 29]. Using common entomological sampling methods, both *An. gambiae s.s* and *An. funestus* mosquitoes are now found only in very small numbers.

LLINs and IRS compounds

Four net types (three LLINs and one non-insecticidal net) and three IRS insecticides of different classes (one organochloride, one synthetic pyrethroid, and one organophosphate) were used. The LLINs included Olyset® nets (manufactured by A-Z, Tanzania), PermaNet 2.0® nets (Vastergaard, Switzerland) and Icon Life® nets (Bestnet Europe ltd, Denmark). Olyset® nets are made of polyethylene netting (150 denier), impregnated during manufacture with synthetic permethrin at 2% w/w (equivalent to 1000mg of active ingredient/m²). PermaNet 2.0® is a 100%-polyester net (100 denier), coated with 55-62mg of synthetic deltamethrin/m², resulting in insecticide concentrations of approximately 0.14% w/w. Icon Life® is also a polyethylene net and is impregnated during manufacture with synthetic deltamethrin at 0.2% w/w (\approx 65mg of active ingredient/m²). The IRS treatments included 2g/m² DDT wettable powder (AVIMA, South Africa), 0.03g/m² lambda-cyhalothrin capsule suspension, (Syngenta, Switzerland) and 2g/m² pirimiphos-methyl emulsified concentrate, also known as actellic (Syngenta, Switzerland).

The IRS compounds and all the LLINs, except Icon Life®, have been approved by WHO for malaria vector control [26]. DDT (an organochloride) and lambda cyhalothrin (a synthetic pyrethroid) are both commonly used for IRS in Africa, and

together with pirimiphos-methyl (a WHO approved organophosphate), they represent a diversity of common insecticide classes currently applicable for vector control in the continent [26]. Similarly, PermaNet 2.0® and Olyset® nets are the most widely used LLINs in Africa. In 2010 alone, approximately 60 million Olyset® nets were manufactured (including 30 million manufactured in Tanzania), and about 75% of all these were scheduled to be used in Africa (Dr. John Lucas, Personal Communication).

Experimental huts and mosquito traps

The IRS compounds, LLINs and their combinations were comparatively evaluated using specially designed huts, referred to as the Ifakara experimental huts. Details of this hut design, and all entomological practices associated with its use, have been described elsewhere [25]. In summary, the Ifakara experimental huts have similar average dimension and shape as local village houses used in the study area. They have galvanized iron frames and corrugated iron roofs, overlaid with grass thatch to regulate temperatures. The undersides of the roofs were covered with ceilings made of traditionally-woven grass mats, locally known as *Mikeka* to simulate thatch. The walls are constructed using canvas on the outside and are lined on the inside with removable wood panels that are coated with clay mud to simulate mud walls. These mud panels and *Mikeka* ceilings are sprayed with insecticides, and can be removed and incinerated at the end of each experiment, then replaced in readiness for any new tests. Each hut has one door, four windows and open eave spaces all round [25].

To study behavioural and physiological responses of mosquitoes in and around the experimental huts, each hut was fitted with interception traps as follows: eight exit traps were fitted on eave spaces (eave traps), and four window traps were fitted onto all the windows (window traps), so as to catch mosquitoes exiting the huts. The eave

traps were interspersed, so that there were adequate spaces between them to allow in mosquitoes attempting entry on all four sides of the huts. These open spaces were fitted with baffles, i.e. netting barriers facing the inside of the huts but slanting upwards at approximately the same angle as the roofs. The baffles allow mosquitoes to enter, freely but restrict exit of those mosquitoes through the same openings, meaning that mosquitoes once inside the huts could exit only through the spaces fitted with exit traps. A detailed description and illustrations of both the traps and the baffles can also be found in Okumu *et al.*, [25].

Study design

We set up nine experimental huts in a line (20-50 metres apart), at the edge of the study village, such that the huts were between the main mosquito aquatic habitats (a contiguous set of small perennially swampy rice fields) and human settlements. For ease of reference, the huts were assigned numbers 1-9 starting with the northernmost to the southernmost hut. Two male volunteers, aged between 18 and 35 years, were assigned to each hut for the duration of the study and slept under intact nets in each of the huts each night.

The huts were first stratified by identifying six huts for IRS (huts 1, 3, 5, 7, 8 and 9), and three huts to remain unsprayed (huts 2, 4, and 6). Each of the six IRS huts was then randomly assigned to be sprayed with any one of the 3 candidate IRS insecticides (such that there were 2 randomly assigned huts sprayed with each insecticide). The IRS was applied at the following WHO approved concentrations [26] as follows: 2g/m² emulsified concentrate of actellic in huts 1 and 8, 2g/m² DDT wettable powder in huts 3 and 5, and 0.03g/m² lambda cyhalothrin capsule suspension in huts 7 and 9. By spraying more than one hut with the same compound, and also by

interspacing the IRS huts with the unsprayed huts, we were attempting to also minimise potential differences in mosquito catches between the huts; given that our baseline studies had indicated that while mosquito catches in all 9 huts were generally similar, huts 1 and 9 tended to have more mosquitoes than the rest [25].

All insecticides were diluted in water and the spraying was performed using standard Hudson Expert[®] sprayers on both the hut walls and ceilings. To avoid contamination, the interception traps and baffles for the IRS huts were fitted 2 days after spraying, allowing time for the insecticide fumes to settle. Also, all the LLINs used were newly acquired, but were air dried outdoors for twelve hours prior to the start of the experiments to prevent any side effects that may be experienced when nets are freshly opened from the packets.

On the first day of the experiment, the three different LLINs (Olyset[®], PermaNet 2.0[®] or Icon Life[®]) and untreated nets were randomly assigned to the nine Ifakara experimental huts, so that each hut regardless of whether it had been sprayed or not, was fitted with either one type of LLINs or untreated nets. In the subsequent days, the nets were rotated daily to different huts as shown in Table 1, ensuring that at any given time, the different LLINs were either coupled with IRS insecticides in the respective huts or the nets were used alone in the unsprayed huts. This experimental design also ensured that in the course of these rotations, there were nights when some of the unsprayed huts ended up with just the untreated nets, thereby constituting the experimental controls, against which effects of the other treatments (i.e. LLINs alone, IRS alone or LLIN/IRS combinations) could be compared. Two nets were used per hut, one per volunteer.

On a 4-day complete block (Table 1), there were 3 replicates of the controls, 3 replicates during which the unsprayed huts had each of the 3 LLIN types on their own

(i.e. LLINs alone), 2 replicates during which the huts had each of the IRS compounds with just the untreated net (i.e. IRS alone) and 2 replicates during which each IRS compound was combined with each of the LLINs (LLIN/IRS together). The experiments were performed on 5 consecutive days each week, so that the volunteers and the technicians could rest every Saturday and Sunday of the week and so that the blocks were not always rotated on the same day of the week. Over the course of the entire experiment the treatment blocks were balanced so that there were equal numbers of each treatment, in a full-factorial split-plot design with repeated measures [30]. Though the LLINs were randomly assigned to the huts initially, their movement between huts each night was not completely randomised in order to simplify the experiment for the field staff, thus avoiding human error in daily allocation of treatments (Table 1).

Table 1: A 4-day roster for allocating LLINs and untreated nets in experimental huts sprayed with different IRS insecticides* during spray round I

| IRS | Hut 1 (Actellic) | Hut 2 (No IRS) | Hut 3 (DDT) | Hut 4 (No IRS) | Hut 5 (DDT) | Hut 6 (No IRS) | Hut 7 (Lambda cyhalothrin) | Hut 8 (Actellic) | Hut 9 (Lambda cyhalothrin) |
|-------|---------------------|-------------------|----------------|-------------------|----------------|-------------------|----------------------------------|---------------------|----------------------------------|
| Day 1 | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® |
| Day 2 | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® |
| Day 3 | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® |
| Day 4 | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated |

*Untreated nets inside unsprayed huts constituted *controls*. Two nets were used per hut, one for each of the two volunteers sleeping in each hut.

Mosquito collection

Experiments were conducted from 19.00 hours to 07.00 hours each night. Mosquitoes were collected using the exit traps on eaves and windows and also through indoor resting collections from the inside surfaces and floors of the huts. Mosquitoes found in the exit traps were removed every 4 hours nightly i.e. at 23.00hrs, 03.00hrs and at 07.00hrs, to ensure that those mosquitoes attempting to exit the huts did not remain unnecessarily confined, thus potentially being exposed to the insecticides for a longer period than would occur in local houses with a similar open design.

To ensure that all mosquitoes inside the huts were removed, the morning collection was performed in two steps as follows: first the collectors emptied all the exit traps, collected all mosquitoes resting on the inside hut surfaces and also retrieved any dead mosquitoes found lying on the floors. The collectors then stayed outside the hut for 10 minutes, after which they went back in and repeated the procedure, thus maximising chances that even those mosquitoes that may have been flying around or missed during the initial collection were now captured. In addition to these 3 main collections per night (i.e. 23.00hrs, 03.00hrs and 07.00hrs), we also collected mosquitoes that entered and rested within the huts during the day or just before the experiments started, by emptying the traps every evening, starting at 18.30hrs, before the volunteers went into the huts at 19.00hrs. Since the LLINs rotated between huts and were set up each morning, those mosquitoes from the evening collections were considered to have been affected by the test interventions in the same way as those mosquitoes entering the huts at night and were added to the nightly totals.

All collected mosquitoes (dead and live) were kept in small netting cages (measuring 15cm × 15 cm × 15cm), on top of which 10% glucose solution was provided via soaked cotton wool pads. The mosquitoes were kept for 24 hours inside

a holding room at the same field site where the experimental huts are located. Mean indoor temperatures inside this holding room were $29.1^{\circ}\text{C} \pm 3.0^{\circ}\text{C}$ during the day and $26.7^{\circ}\text{C} \pm 2.3^{\circ}\text{C}$ at night, while mean relative humidity was $70.6\% \pm 17.9\%$ during the day and $75.7\% \pm 13.7\%$ at night. After the 24-hour holding period, dead and live mosquitoes were segregated. Live mosquitoes were killed with ethyl acetate after which each group was sorted by taxon and counted.

Malaria vectors, *An. gambiae* s.l and *An. funestus* s.l, together with all other *Anopheles* mosquitoes found during the study were first distinguished morphologically from the Culicine mosquito genera, *Culex* and *Mansonia* species. A sub-sample of one dead and one live *An. gambiae* s.l mosquitoes per hut per night per collection period, were randomly selected for further identification using ribosomal DNA-polymerase chain reaction (PCR) [31] to distinguish between *An. arabiensis* and *An. gambiae sensu stricto*, the two morphologically indistinguishable sibling species known to be in the study area [28, 29]. Similarly, *An. funestus* s.l were molecularly analysed using PCR to determine sibling species within the group. Given that there was only a small number of *An. funestus* s.l mosquitoes caught during the entire study duration, all of them were analysed without any sub-sampling. All the molecular analysis work was performed at Ifakara Health Institute, Tanzania.

Spray rounds

This study was conducted in two spray rounds, the first round being four months long (May 2010 to August 2010) and the second being six months (November 2010 to April 2011). To limit complications of having to rotate treated and untreated mud panels and ceilings between huts, the huts with IRS treatments were fixed for the entire duration of each spray round, and instead only the LLINs were rotated.

However, all the mud panels and *Mikeka* ceilings, and an inner plastic sheeting usually placed under the sprayed surfaces to ensure that the huts are not contaminated, were removed and incinerated at the end of the first round, and were replaced with fresh material prior to starting the second round, which was three months after the end of the first round. The two rounds were mostly similar except for some incremental improvements introduced in round II. The methodological aspects already described in the sections above match the second round of the study, but all the differences in the first round relative to the second are outlined below and in other relevant sections.

First, unlike in the second round, where the IRS insecticides were randomly assigned to the preselected IRS huts, the procedure in the first round was that both the IRS insecticides and the LLINs were systematically assigned to the preselected IRS huts (Table 2). Second, to approximate WHO guidelines [26] regarding the periods after which IRS houses should be re-treated (i.e. 2-3 months for actellic, 3-6 months for lambda cyhalothrin and 6-12 months for DDT), experiments were conducted over 4 months in round one and over 6 months in round two. Third, the two-step procedure for mosquito collections in the morning was introduced in round two following observations in round one that the original one-step procedure was not adequately exhaustive and that some mosquitoes were being left behind by the collectors. Fourth, to minimise any likelihood that the insecticides sprayed on the walls or the chemical particles on the nets would be agitated and blown by wind or air currents and that these insecticidal treatments would accumulate in the exit traps, the second spray round involved blocking 3 of the 4 windows in all huts during the day, using a piece of canvas cut to fit the window sizes. The canvas was however removed in the evenings so that the window traps could be used normally during the night. This and other important entomological procedures used have been described elsewhere [25].

Table 2: A 4-day roster used in the second round of the study (as different from the first round shown in Table 1), for allocating LLINs and untreated nets in experimental huts sprayed with different IRS insecticides[‡].

| IRS | Hut 1 | Hut 2 | Hut 3 (Lambda cyhalothrin) | Hut 4 (No IRS) | Hut 5 (Actellic) | Hut 6 (No IRS) | Hut 7 (DDT) | Hut 8 (Lambda cyhalothrin) | Hut 9 (Actellic) |
|-------|------------|------------|----------------------------------|-------------------|---------------------|-------------------|----------------|----------------------------------|---------------------|
| Day 1 | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® |
| Day 2 | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® |
| Day 3 | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® |
| Day 4 | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated | PermaNet® | Icon Life® | Olyset® | Untreated |

[‡]Untreated nets inside unsprayed huts constituted *controls*. Two nets were used per hut, one for each of the two volunteers sleeping in each of the huts.

Sampling for analysis of insecticide residues on walls, ceilings and nets

To determine whether the required quantities of insecticides had been correctly sprayed onto hut surfaces, material samples were collected from the walls and ceilings of the experimental huts. Sample squares were also cut from the different nets, to estimate the insecticide quantities at the start of the experiments. The sampling procedure was different for the two spray rounds of the study, but was each time in line with WHO guidelines [32].

In the first round, the sampling was as follows: using a flat-tip spatula, soil was gently scrapped from a small randomly selected area measuring 20cm², on the inside surfaces of any 2 randomly selected walls of each sprayed hut. The person doing this was always different from the person who had sprayed the huts in the first place. At the same time, 2 small pieces (20cm² each) were snipped from 2 randomly selected positions on the *Mikeka* ceilings of each sprayed hut. This way, we had 4 samples collected from each hut, i.e. 2 soil samples from the mud walls and 2 samples from the ceilings. Similarly, samples were collected from the nets, by snipping a 15cm × 15cm area from each of the four sides of all the nets, including the untreated net being used in the study. The net cuttings were obtained from the bottom parts of the nets (i.e. from parts which would normally be tacked under mattresses when the nets are in use), so as not to leave the net visibly holed and the volunteer exposed. The sample collections were performed at the beginning of the experiment immediately after the experimental huts were sprayed and the nets unbundled and air dried.

The soil samples were thoroughly shaken to homogenously mix the chemical residues with the soil. Both the ceiling and soil samples were then weighed, after which a sub-sample (weighing 1g from the soil samples and 1 to 2g from the ceiling samples) was taken and stored in 4ml glass vials. The glass vials were labelled to

indicate the hut from which the samples had been collected, the insecticide sprayed on the huts and the type of surface (walls or ceilings). Both the glass vials and the net cuttings were then carefully wrapped in aluminium foil and shipped to laboratory at London School of Hygiene and Tropical Medicine (LSHTM), where they were analysed by way of high performance liquid chromatography (HPLC) to identify and quantify the insecticide residues in them. Samples were stored at 4°C to prevent degradation of insecticidal residues.

Sampling for residues in the second round of the study was as follows: we attached 4 pieces of Watman[®] filter papers (each measuring 44cm²) onto each of the walls and another 4 pieces of the same size onto the ceilings of the huts, prior to spraying [32, 33]. After the spraying was completed and the hut surfaces dried, one piece of filter paper was randomly selected from each side of the hut walls and another 2 pieces selected from the ceiling (totalling to 4 wall pieces and 2 ceiling pieces per hut). During spraying, it was possible that the spray man sub-consciously sprayed more insecticide solution onto the filter papers, than onto the other hut surfaces. The purpose of using multiple filter papers on each wall and on the ceilings and then randomly selecting a sample of the filter papers, was therefore to reduce the effects of this subconscious tendency. The selected filter papers were carefully removed, folded and kept in petri-dishes, which were then wrapped in aluminium foil. With regard to the nets, sampling was done by snipping 20cm² pieces as described above for round 1 of the study. The petri-dishes were wrapped in aluminium foil to avoid any degradation of the insecticides, and the samples immediately transported to LSHTM for HPLC, where they were stored at 4°C before analysis.

Protection of participants and ethical approval

Participation of volunteers in all the experiments was voluntary, even though all participants were paid nightly wages to compensate for their time. After full explanation of purpose and requirements of the studies as well as the risks and benefits of participation, written informed consent was obtained from each volunteer prior to the start of all experiments. While inside the experimental huts, the volunteers slept under intact bed nets as a basic protection against mosquito bites. They were also provided with long sleeved, hooded jackets and gumboots, so as to provide additional protection from bites whenever the volunteers stepped outside the nets to collect mosquitoes. In addition, the volunteers were provided with access to weekly diagnosis for malaria parasites, using rapid diagnostic test kits and treatment with the current first-line malaria drug (artemether-lumefantrine) if they had malaria. Perceived adverse effects from exposure to insecticides were monitored by the study co-ordinator and volunteers were free to leave the study at any time. Ethical approval for this study was granted by the Institutional Review Board of the Ifakara Health Institute (IHRDC/IRB/No.A019), the Tanzania National Institute of Medical Research (NIMR/HQ/R.8aNo1.W710) and the London School of Hygiene and Tropical Medicine Ethical Review Board (Ethics Clearance No. 5552).

Statistical analysis

Power calculation: baseline data [25] were used to calculate the number of replicates required to observe a 23% difference in mosquito hut entry relative to the control, chosen as the average effect size observed from LLINs [25] using a non-central two-sided t-distribution in STATA 11.0 (StataCorp) [34]. Deterrence was selected as the outcome to calculate power, given that it is the smallest effect generally observed in

experimental hut trials, and mortality was considered as generally exceeding 50%, so as to avoid under-powering of the study. Power calculations showed that a minimum of 64 replicates were required to see a significant difference in the mean number of mosquitoes in huts with 95% confidence and 80% power.

Analysis of number of mosquitoes entering huts: data were analysed using R statistical software version 2.13.0, with the statistical library lme4 [35]. The nightly total number of mosquitoes of each taxon caught inside the huts or in the exit traps was first calculated by summing live and dead mosquitoes from the respective huts, for each collection period. The mosquito catches were then aggregated to obtain the total catches per night per hut. The total number of mosquitoes of each taxon was compared between huts having the various insecticidal treatments (IRS, LLINs or IRS/ LLINs combinations) and the controls (untreated nets in unsprayed huts).

The aggregated data was fitted to a generalized linear mixed effects model (GLMM), with Poisson errors, a log link and a random factor for each individual data point (i.e. a log normal Poisson model) to account for over-dispersion in the count data. Data was analysed as a function of the three fixed factors, treatment (insecticidal combinations), time (number of months since the start of the experiment), and day order (a variable representing the fact that our net rotations were conducted on consecutive nights between Mondays and Fridays, but not on Saturdays and Sundays).

Random factors in the model included hut and day of mosquito collection. Satisfactory model fits were confirmed using a Wald function test, and the estimated mean number of mosquitoes entering the different huts, and their

95% confidence intervals, were calculated as exponentials from the coefficients generated from the generalised linear mixed model. This way it was possible to determine whether huts with different insecticidal treatments had significantly higher or lower catches than the controls whilst accounting for data structure and design factors that might influence the results.

Mosquito mortality: data was analysed using R statistical software version 2.13.0 with the statistical package *lme4* [35]. 24-hour mortality associated with the different insecticidal applications was analysed in two different ways: 1) by considering the proportions of mosquitoes entering individual huts that died in each occasion, a measure suitable for estimating personal household level protection of humans sleeping in the respective treated houses and 2) by considering the actual numbers of mosquitoes that were killed by the different treatments relative to the controls, a measure suitable for estimating community level mass protection that such treatments can confer.

To compare the proportional mosquito mortalities, the data was fitted to GLMMs with binomial errors and a *logit* link and analysed as a function of insecticidal combinations, month and day order, including hut and date as random factors. A Wald function test was used to assess the best model fit. Due to high mortalities in the controls, data from the second spray round was corrected using Abbots formula for corrected mortality [36]. To compare the actual number of mosquitoes killed by the different treatments, Poisson-lognormal GLMMs with the same fixed and random factors as above were applied to the data.

Timing of mosquito exit: This analysis was performed using SPSS version 16 (SPSS inc.) using linear regression of log transformed mosquito count data. To assess whether the insecticidal treatments affected the times when mosquitoes naturally exited the huts, the mosquito catches in the exit traps at the different periods of the night (6pm collections, 7pm - 11pm, 11pm - 3am and 3am - 7am), were computed as percentages of the total exit trap catches each night, in the different huts. Chi-square analysis was performed to determine if any of the observed percentage increases in early exit were significant relative to the controls.

Finally, to assess whether the huts that had more mosquitoes were also the huts that had greater proportions dead, i.e. whether the huts design was letting out mainly live mosquitoes, we explored statistical correlations between the total catches and percentage mortalities among catches of different species. To accomplish this, linear regression analysis was performed on the log transformed *An. arabiensis* catches and proportional mortality computed for these species.

Results

Molecular analysis of mosquitoes

PCR analysis of the *An. gambiae* s.l samples collected during the first spray round showed that among the 445 successful individual mosquito DNA amplifications, 98.7% were *An. arabiensis* (n = 439) and 1.3% *An. gambiae* s.s (n = 6). All of the 275 *An. funestus* complex mosquitoes collected over the 4 month experimental duration were subjected to molecular analysis, which resulted in 233 successful DNA amplifications. It was found that 96.6% of these (n = 225) were *An. funestus sensu stricto*, while the remaining 3.43% (n = 8) were *An. rivolurum*.

In the second spray round, PCR analysis was done on 782 *An. gambiae* s.l samples, among which there were 720 successful individual mosquito DNA amplifications. It was found that 95.7% were *An. arabiensis* (n = 689) and 4.3% were *An. gambiae* s.s (n = 31). No molecular identification data was obtained on *An. funestus* during the second spray round.

Number of mosquitoes caught in experimental huts with different treatments

Tables 3 and 4 show the summary statistics, including model estimated means (and 95% confidence intervals) of *An. arabiensis* mosquitoes that were caught in the different experimental huts during the two spray rounds. The actual catches are represented in the tables by medians and sums of different mosquito taxa. None of the nets demonstrated a pronounced deterrent effect in either spray round. In the first spray round (Table 3), only PermaNet® was deterrent, reducing the catches of *An. arabiensis* by 30.3% ($z = -2.192$, $P = 0.028$) relative to untreated nets. On the contrary, huts fitted with Icon Life® nets had significantly more mosquitoes than the controls in round I ($z = 2.74$, $P = 0.006$).

In round I there was a 43.0% reduction of *An. arabiensis* catches where DDT was used alone ($z = -2.023$, $P = 0.043$), and a non-significant reduction of 37.7% in huts where DDT was used together with PermaNet® nets ($z = -1.808$, $P = 0.071$). However in round II, which was conducted over 6 months this deterrent effect was not evident. In the second spray round (Table 4), none of the treatments reduced malaria mosquito catches relative to the controls ($P > 0.05$). Unlike in the first round, relative increases in number of *An. arabiensis* mosquitoes were observed with all treatments, except PermaNet® nets used alone ($z = 0.935$, $P = 0.351$), actellic IRS combined with untreated nets ($z = 1.495$, $P = 0.135$), or DDT with untreated nets ($z = 1.863$, $P =$

0.063). In both spray rounds addition of Olyset® or Icon Life® LLINs into huts with the different IRS compounds tended to increase *An. arabiensis* catches relative to the different IRS compounds on their own, whereas the addition of PermaNet® increased mosquito numbers, but to a smaller extent (Table 4).

No significant differences were observed in *Culex* mosquito catches other than decrease when actellic IRS was combined with Olyset® ($z = -2.199$, $P = 0.028$) or PermaNet® nets ($z = -2.566$, $P = 0.010$) in round I and increases when actellic was used with untreated nets ($z = 2.359$, $P = 0.018$) or in combination with Olyset® nets ($z = 2.795$, $P = 0.005$), and a decrease when lambda cyhalothrin was combined with Olyset® nets ($z = -2.028$, $P = 0.043$) in round II. We also observed no difference in catches of *Mansonia* mosquitoes between huts with the various treatments relative to the control ($P > 0.05$) apart from a decrease when using Olyset® nets alone ($z = -3.267$, $P = 0.001$), or PermaNet® nets alone ($z = -2.088$, $P = 0.037$) in Round II.

The number of mosquitoes caught was greatly varied by month of study, coinciding with the progression of the wet season in the study area. For example during the second spray round, *An. arabiensis* catches was higher in the second month (December 2010), third month (January 2011) and fourth month (February 2011) compared to the first month (November 2010) and sixth month (April 2011) (Figures 1A & B). This trend was the same regardless of whether we considered the data from experimental huts where volunteers used only the different net types (Figure 1A), or data from experimental huts where only the different insecticides were used (Figure 1B).

Table 3: Summary statistics table for number of mosquitoes collected each night during the **first spray round**. Table showing the median number (and inter-quartile ranges (IQR)), the model estimated means with 95% confidence intervals (*Anopheles arabiensis* only), and the sum of mosquitoes caught per night in experimental huts fitted with different IRS and LLIN treatments^{*}

| IRS/LLIN combinations | Anopheles arabiensis | | | | | Culex species | | | Mansonia species | | |
|----------------------------------|----------------------|--------------|-----------|-----------------|--------------|---------------|-------------|------|------------------|------------|------|
| | Median | IQR | Sum (n)^ | Model estimates | | Median | IQR | Sum^ | Median | IQR | Sum^ |
| | | | | *Mean | 95%CI | | | | | | |
| Untreated nets only** | 66.5 | 20.3 - 103.8 | 4596 (60) | 60.5 | 39.9 - 91.9 | 26.0 | 9.0 - 62.3 | 2388 | 9.5 | 4.0 - 21.0 | 802 |
| Olyset alone | 89.0 | 62.3 - 128.5 | 6047 (60) | 60.2 | 43.7 - 82.9 | 26.5 | 10.0 - 65.8 | 2701 | 11.0 | 3.3 - 15.8 | 743 |
| PermaNet alone | 67.0 | 46.8 - 95.0 | 4420 (60) | 42.2 | 30.6 - 58.3 | 28.0 | 10.3 - 56.3 | 2257 | 7.0 | 4.0 - 16.0 | 627 |
| Icon Life | 79.0 | 47.3 - 130.0 | 6492 (60) | 87.7 | 67.3 - 114.4 | 23.0 | 11.3 - 66.8 | 2434 | 13.5 | 4.5 - 23.0 | 910 |
| Actellic only | 89.0 | 57.5 - 162.8 | 4512 (40) | 57.8 | 33.4 - 100.0 | 25.5 | 10.5 - 51.5 | 1437 | 13.0 | 6.3 - 27.3 | 669 |
| Actellic and Olyset | 119.5 | 71.3 - 175.5 | 5466 (40) | 95.8 | 57.5 - 159.7 | 27.0 | 9.8 - 71.3 | 1555 | 10.0 | 3.0 - 16.0 | 496 |
| Actellic and PermaNet | 87.5 | 60.3 - 139.3 | 4691 (40) | 74.0 | 44.0 - 124.6 | 22.5 | 11.0 - 57.5 | 1438 | 13.0 | 7.3 - 23.0 | 656 |
| Actellic and Icon Life | 124.5 | 78.0 - 216.5 | 6022 (40) | 82.0 | 47.5 - 141.5 | 33.5 | 14.5 - 66.5 | 1884 | 13.5 | 7.0 - 30.8 | 800 |
| DDT only | 45.0 | 32.3 - 94.3 | 2605 (40) | 34.5 | 20.0 - 59.5 | 21.5 | 10.3 - 48.3 | 1380 | 10.0 | 3.3 - 15.8 | 414 |
| DDT and Olyset | 74.5 | 45.5 - 102.8 | 3162 (40) | 52.1 | 31.3 - 86.8 | 26.0 | 7.3 - 54.5 | 1650 | 8.0 | 3.0 - 15.0 | 366 |
| DDT and PermaNet | 55.5 | 38.3 - 74.8 | 2728 (40) | 37.7 | 22.5 - 63.0 | 24.5 | 10.3 - 44.8 | 1530 | 6.5 | 3.3 - 17.5 | 414 |
| DDT and Icon Life | 94.0 | 62.5 - 128.0 | 4017 (40) | 60.8 | 35.4 - 104.3 | 22.5 | 10.0 - 48.5 | 1709 | 10.0 | 4.0 - 14.8 | 422 |
| Lambda cyhalothrin alone | 82.0 | 60.8 - 137.8 | 4212 (40) | 58.1 | 34.5 - 98.1 | 34.0 | 9.5 - 67.3 | 1673 | 9.5 | 6.0 - 15.0 | 533 |
| Lambda cyhalothrin and Olyset | 99.0 | 61.0 - 186.8 | 5323 (40) | 91.2 | 54.2 - 153.6 | 29.0 | 7.3 - 51.8 | 1355 | 6.5 | 2.0 - 12.5 | 361 |
| Lambda cyhalothrin and PermaNet | 85.5 | 41.5 - 141.0 | 3931 (40) | 52.3 | 31.0 - 88.2 | 31.0 | 9.3 - 64.8 | 1596 | 7.0 | 3.0 - 17.0 | 494 |
| Lambda cyhalothrin and Icon Life | 106.0 | 59.3 - 174.5 | 5434 (40) | 85.3 | 50.9 - 143.0 | 28.5 | 7.0 - 56.3 | 1477 | 11.5 | 5.0 - 23.0 | 598 |

* Refers to the model estimated means as calculated in R.

[^] The term 'n' refers to total number of replicates

[§] The number of replicates (n) was the same as for *An. arabiensis*

**Controls refer to unsprayed huts in which volunteers used untreated nets

Table 4: Summary statistics table for number mosquitoes collected each night during the **second spray round**. Table showing the median number (and inter-quartile ranges (IQR)), the model estimated means with 95% confidence intervals (*Anopheles arabiensis* only), and the sum of mosquitoes caught per night in experimental huts fitted with different IRS and LLIN treatments

| IRS/LLIN combinations | Anopheles arabiensis | | | | Culex species | | | Mansonia species | | | |
|----------------------------------|----------------------|--------------|------------|-----------------|---------------|--------|-------------|------------------|--------|------------|------|
| | Median | IQR | Sum (n)^ | Model estimates | | Median | IQR | Sum^ | Median | IQR | Sum^ |
| | | | | *Mean | 95%CI | | | | | | |
| Untreated nets only** | 64.0 | 36.5 - 95.0 | 7181 (90) | 49.0 | 33.7 - 71.2 | 22.0 | 11.0 - 39.5 | 2461 | 5.0 | 3.0 - 8.0 | 537 |
| Olyset alone | 84.0 | 43.0 - 145.8 | 9789 (90) | 65.6 | 50.4 - 85.3 | 23.0 | 10.0 - 39.3 | 2498 | 3.0 | 1.0 - 5.3 | 380 |
| PermaNet alone | 61.0 | 41.5 - 118.3 | 8240 (90) | 55.5 | 42.7 - 72.3 | 23.0 | 10.0 - 41.5 | 2544 | 3.5 | 1.0 - 6.3 | 412 |
| Icon Life | 105.0 | 57.0 - 164.3 | 11279 (90) | 75.7 | 61.3 - 93.4 | 22.5 | 13.8 - 43.3 | 2668 | 6.0 | 3.0 - 11.0 | 703 |
| Actellic only | 85.0 | 52.3 - 141.8 | 6751 (60) | 63.5 | 45.2 - 89.1 | 33.5 | 14.5 - 65.8 | 3102 | 9.0 | 3.3 - 13.0 | 652 |
| Actellic and Olyset | 136.0 | 74.8 - 208.3 | 9988 (60) | 108.3 | 77.6 - 151.2 | 33.5 | 16.5 - 74.0 | 3384 | 6.0 | 3.0 - 9.8 | 437 |
| Actellic and PermaNet | 94.5 | 59.0 - 191.3 | 7978 (60) | 78.9 | 56.3 - 110.6 | 30.0 | 17.0 - 62.3 | 3032 | 7.0 | 3.3 - 11.8 | 518 |
| Actellic and Icon Life | 144.5 | 72.5 - 197.5 | 9621 (60) | 102.8 | 73.7 - 143.5 | 37.5 | 16.3 - 59.5 | 3023 | 9.0 | 5.0 - 17.0 | 722 |
| DDT only | 67.0 | 38.3 - 107.8 | 4983 (60) | 69.1 | 48.1 - 99.2 | 23.0 | 12.3 - 46.3 | 1828 | 4.0 | 2.0 - 8.0 | 365 |
| DDT and Olyset | 76.0 | 51.3 - 129.5 | 6053 (60) | 81.8 | 59.1 - 113.1 | 25.5 | 10.3 - 40.8 | 1894 | 3.0 | 1.0 - 5.8 | 256 |
| DDT and PermaNet | 72.0 | 41.3 - 135.0 | 5528 (60) | 75.7 | 54.7 - 104.8 | 27.0 | 10.3 - 40.5 | 1909 | 4.0 | 2.0 - 6.8 | 271 |
| DDT and Icon Life | 82.0 | 48.5 - 148.5 | 6176 (60) | 80.6 | 56.2 - 115.6 | 29.0 | 15.0 - 43.8 | 1925 | 4.0 | 2.3 - 9.0 | 438 |
| Lambda cyhalothrin alone | 100.5 | 51.3 - 178.5 | 7535 (60) | 70.9 | 49.4 - 101.8 | 20.5 | 10.3 - 38.0 | 1950 | 7.5 | 4.0 - 13.0 | 620 |
| Lambda cyhalothrin and Olyset | 115.5 | 65.5 - 207.0 | 8947 (60) | 79.0 | 57.1 - 109.2 | 23.0 | 9.8 - 34.0 | 1916 | 5.0 | 2.0 - 9.8 | 438 |
| Lambda cyhalothrin and PermaNet | 100.5 | 58.3 - 173.8 | 7622 (60) | 73.0 | 52.7 - 101.1 | 22.0 | 9.5 - 37.8 | 2018 | 6.0 | 3.0 - 12.0 | 548 |
| Lambda cyhalothrin and Icon Life | 120.0 | 71.8 - 243.5 | 9784 (60) | 90.5 | 63.1 - 129.7 | 23.5 | 9.0 - 34.8 | 1981 | 8.0 | 5.0 - 15.0 | 706 |

* Refers to the model estimated means as calculated in R.

[^] The term 'n' refers to total number of replicates

[§] The number of replicates (n) was the same as for *An. arabiensis*

**Controls refer to unsprayed huts in which volunteers used untreated nets

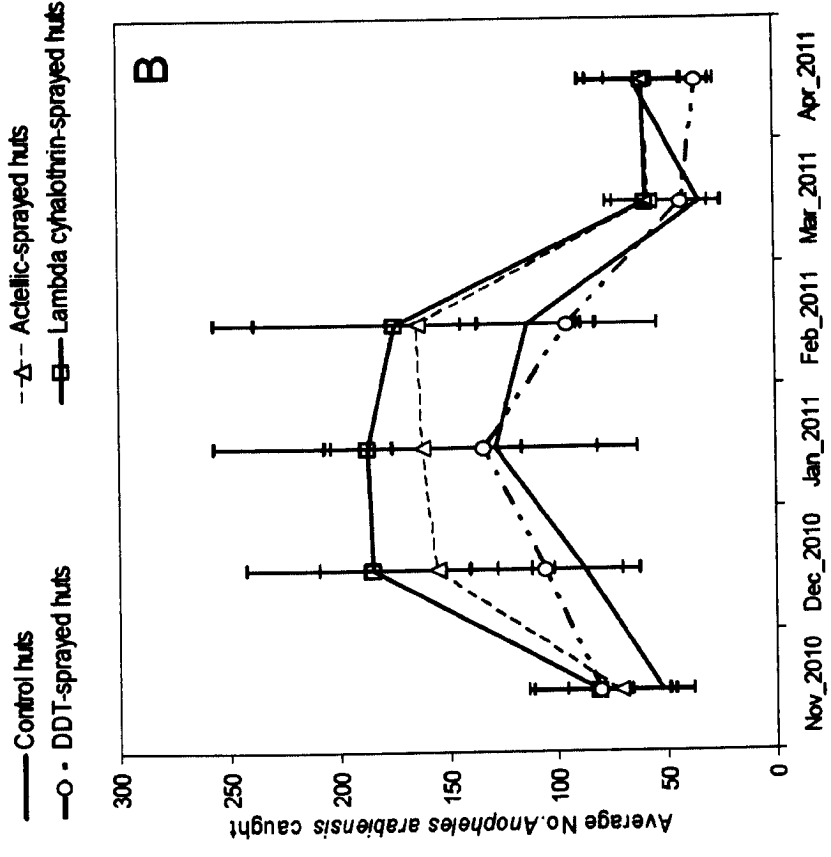
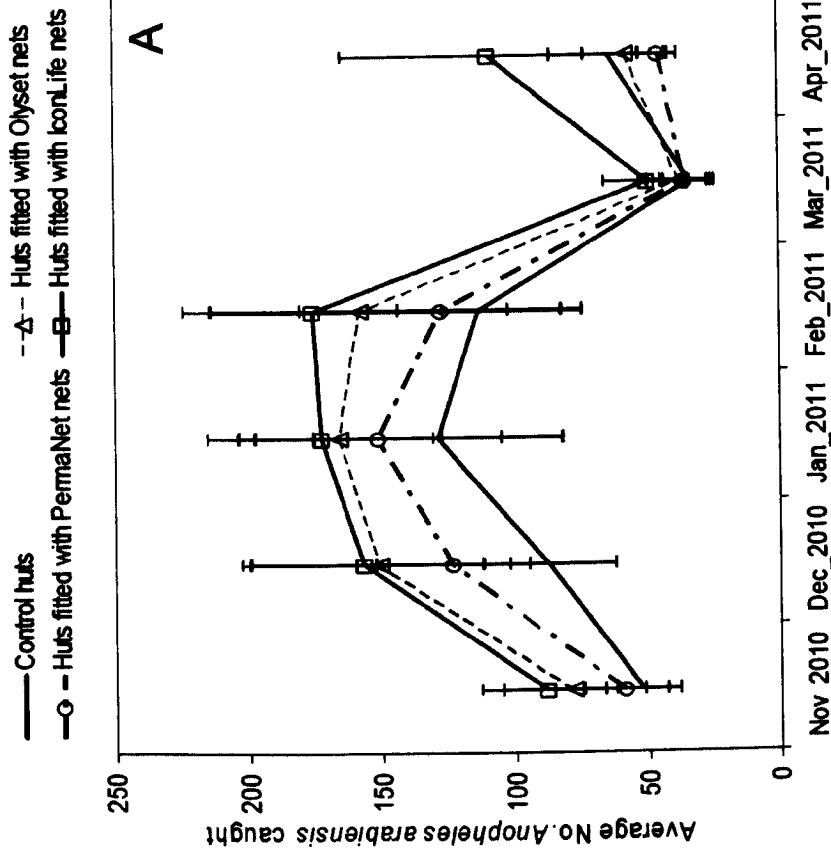


Figure 1: Seasonal variation in the average number of *Anopheles arabiensis* mosquitoes caught per hut per night during the second spray round. Figure 1A represents data from experimental huts where the human volunteers used only the different net types, while Figure 1B shows data from experimental huts the different IRS chemicals were the only treatments. The Y-error bars refer to 95% confidence intervals.

Proportion of dead mosquitoes caught in experimental huts with different LLIN and IRS treatments

Tables 5 and 6 show the summary statistics, including model estimated mean percentages (and 95% confidence intervals) of *An. arabiensis* mosquitoes that were caught in the different experimental huts during the two spray rounds. The median % mortality remained consistent between the two rounds and the relative effects of treatment combinations remained similar between rounds with addition of actellic IRS consistently inducing greatest additional mortality, while addition of Olyset® nets consistently inducing lowest additional mortality. However, mortality was generally higher in round II than in round I. In the first spray round, all the tested insecticidal applications except DDT combined with Olyset® nets ($z = 1.593$, $P = 0.111$) and Olyset® nets ($z = 1.388$, $P = 0.165$) when used alone significantly increased the percentage mortalities of *An. arabiensis*, relative to the controls ($P < 0.05$).

The most toxic net in round 1 was PermaNet®, which when used alone, killed 19.6% (11.8% - 32.5%) of the vectors ($z = 2.142$, $P = 0.032$), while the most toxic IRS compound was actellic, which when used alone killed 46.8% (27.0% – 81.0%) of the vectors ($z = 4.664$, $P < 0.001$). Overall, the most toxic combination was actellic IRS combined with PermaNet® nets (estimated mean mortality of *An. arabiensis* being 53.5% (31.3% - 91.5%)) over the 4 month test period ($z = 7.189$, $P < 0.001$). Compared to any of the LLINs when used alone, only actellic IRS (but not DDT or lambda cyhalothrin) increased proportions of *An. arabiensis* mosquitoes killed during this first spray round (Table 5). However, when median percentage mortality was calculated for different months, we observed short-lived enhancement of benefits in the first two months, during which addition of all the IRS compounds except DDT increased the proportions of dead mosquitoes relative to just the LLINs alone (Table

7). For example, during the first month after spraying, actellic IRS increased median mortality of malaria vectors by 20% when added to huts having Olyset® nets, by 22% when added to huts having PermaNet® nets, and by 29% when added to huts having Icon Life® nets.

On the other hand, relative to IRS alone, there was mostly no apparent additional mortality as a result of introduction of LLINs (Table 5), but again we observed some short-lived protective benefits when the data was broken down by month (Table 7). For example, in the first month, DDT and untreated nets killed 9.8% of *An. arabiensis*, while DDT and PermaNet® killed up to 17.3%. Also, actellic IRS coupled with Icon Life® nets killed 39.2% compared to 27.9% when actellic was used with untreated nets during the same month. Also, it was observed throughout this first spray round, that both PermaNet® and Icon Life® nets were more toxic to *An. arabiensis* than Olyset® nets alone (Tables 5 and 7).

During the second spray round, there was an unusually high mortality in the controls (14.3% (10.8% - 18.6%)), thus the estimates were corrected using Abbots formula [36]. All the treatments killed significantly greater proportions of *An. arabiensis* than the controls ($P < 0.001$). The most toxic LLIN against *An. arabiensis* was Icon Life® nets, which killed 28.5% (24.8% - 32.3%) of all mosquitoes of this species entering the huts. On the other hand, the most toxic of the IRS compounds when used alone was actellic, which killed 37.3% (31.0% - 43.9%) of all *An. arabiensis* entering the huts (Table 6). Addition of PermaNet® or Icon Life® nets but not Olyset® nets tended to increase proportions of mosquitoes dying relative to the IRS alone (Tables 6 and 7). It was observed that incremental toxicity in cases where IRS was added onto any of the LLINs was greatest when actellic was the candidate IRS, but similar effects of the other IRS compounds was marginal (Table 6).

Though, toxicity of all the treatments to *Culex* mosquitoes was evidently much lower than their toxicity to *An. arabiensis*, the data here from both rounds shows that relative to the controls, significantly higher proportions of *Culex* mosquitoes were killed in huts with either actellic or lambda cyhalothrin IRS ($P \leq 0.003$) and in huts with DDT coupled with PermaNet[®] ($z = 3.674$, $P < 0.001$). In the second round increased proportions of *Culex* mosquitoes killed relative to the controls even though the estimated mean proportions of dead mosquitoes were lower than in the case of *An. arabiensis*. Higher proportions of *Mansonia* mosquitoes were killed in huts with DDT based IRS coupled with PermaNet[®] ($z = 3.402$, $P = 0.001$) in round I and similarly in round II all treatments killed significantly higher *Mansonia* proportions than the controls, except where the huts had DDT alone ($z = 1.164$, $P = 0.245$) or DDT combined with Icon Life[®] nets ($z = 0.889$, $P = 0.374$).

Actual number of mosquitoes killed by the different treatments

In addition to computing the proportional mortality among mosquitoes that entered different experimental huts, we estimated and directly compared the actual numbers of mosquitoes killed in huts that had the different insecticidal treatments, relative to the controls. In addition to the percentage mortalities, Tables 5 and 6 both show also the actual total numbers of mosquitoes of different species that were killed. In both spray rounds Icon Life[®] consistently killed a greater number of mosquitoes than other net types both when used singly or in combination, and actellic IRS was the most toxic of the IRS tested. In round I, the huts with actellic and Icon Life, the estimated mean number of malaria mosquitoes killed per night was 28.4 (15.4 -52.2) compared with an estimated mean of 4.8 (3.1 - 7.3) in the controls. In round II, the greatest increase in number of *An. arabiensis* mosquitoes killed relative to controls was

observed in huts sprayed with actellic supplemented with Icon Life[®] nets ($z = 10.415$, $P = 0.001$). The estimated mean number of dead *An. arabiensis* mosquitoes in these huts was 70.2 (57.1 – 105.4) per night compared with 8.1 (6.6 – 12.3) per night in the controls. Similar to the first spray round, actellic combined with Icon life[®] nets killed the largest number of malaria mosquitoes per night during this spray round, followed by actellic coupled with PermaNet[®] nets.

In both rounds there was a significant increase in number of dead mosquitoes found in experimental huts fitted with all treatments except DDT alone ($z = 0.418$, $P = 0.676$), DDT and Olyset[®] nets ($z = 0.482$, $P = 0.630$), DDT and PermaNet[®] nets ($z = 0.792$, $P = 0.428$), and Olyset[®] nets alone ($z = 1.802$, $P = 0.072$) in the first round only.

Table 5: Summary statistics table for percentage number of mosquitoes that died each night during the **first spray round**. Table showing the median percentage (and inter-quartile ranges (IQR)), the model estimated mean percentage with 95% confidence intervals (for *Anopheles arabiensis* only) and actual number of mosquitoes killed (total killed).

| IRS/LLIN combinations | Mortality of <i>Anopheles arabiensis</i> | | | | Mortality of <i>Culex</i> species | | Mortality of <i>Mansonia</i> species | |
|---------------------------------------|--|--------------------|---------|-------------------------------|-----------------------------------|--------------|--------------------------------------|--------------|
| | Median % (IQR) | Model estimates | | Total killed (n) [^] | Median% (IQR) | Total killed | Median% (IQR) | Total killed |
| | | *Mean% (95% CI) | P value | | | | | |
| Untreated nets only** | 7.1 (3.8 - 14.0) | 7.8 (5.1 - 12.0) | | 403 (60) | 1.0 (0.0 - 06.2) | 77 | 16.5 (5.5 - 36.9) | 170 |
| Olyset only | 11.8 (7.1 - 17.2) | 11.2 (06.7 - 18.7) | 0.165 | 709 (60) | 3.9 (0.0 - 08.8) | 121 | 33.3 (06.2 - 50.0) | 285 |
| PermaNet only | 19.5 (13.6 - 26.5) | 19.6 (11.8 - 32.5) | < 0.001 | 844 (60) | 2.4 (0.0 - 09.0) | 87 | 50.0 (39.6 - 70.1) | 343 |
| Icon Life only | 19.0 (12.4 - 27.5) | 15.3 (09.7 - 24.2) | 0.004 | 1028 (60) | 2.7 (0.0 - 11.1) | 111 | 50.0 (29.6 - 62.8) | 444 |
| Actellic and untreated nets | 16.6 (12.1 - 28.7) | 46.8 (27.0 - 81.0) | < 0.001 | 836 (40) | 9.8 (2.6 - 20.4) | 136 | 42.9 (20.4 - 51.1) | 300 |
| Actellic and Olyset | 16.4 (13.1 - 24.9) | 37.0 (22.6 - 60.6) | < 0.001 | 980 (40) | 7.4 (2.3 - 16.7) | 102 | 41.2 (22.2 - 68.0) | 255 |
| Actellic and PermaNet | 29.0 (18.8 - 36.2) | 51.3 (30.7 - 85.6) | < 0.001 | 1196 (40) | 6.9 (2.3 - 15.3) | 98 | 71.8 (53.3 - 79.1) | 433 |
| Actellic and Icon Life | 21.0 (13.3 - 32.2) | 53.5 (31.3 - 91.5) | < 0.001 | 1338 (40) | 3.3 (0.3 - 12.5) | 108 | 56.5 (36.6 - 70.3) | 433 |
| DDT and untreated nets | 14.0 (7.7 - 24.4) | 15.9 (8.9 - 28.4) | 0.017 | 369 (40) | 1.4 (0.0 - 13.3) | 52 | 50.0 (18.8 - 66.7) | 192 |
| DDT and Olyset | 13.2 (8.8 - 17.2) | 12.1 (7.1 - 20.9) | 0.111 | 411 (40) | 3.0 (0.0 - 11.0) | 53 | 46.7 (21.1 - 62.4) | 162 |
| DDT and PermaNet | 17.2 (12.0 - 25.7) | 19.2 (11.1 - 33.1) | 0.001 | 431 (40) | 4.2 (0.0 - 12.9) | 94 | 53.8 (36.7 - 66.7) | 220 |
| DDT and Icon Life | 12.3 (9.3 - 18.6) | 14.3 (8.2 - 25.0) | 0.032 | 581 (40) | 1.8 (0.0 - 08.8) | 69 | 36.1 (20.2 - 50.0) | 165 |
| Lambda cyhalothrin and untreated nets | 14.8 (10.6 - 22.2) | 18.2 (10.7 - 31.0) | 0.002 | 634 (40) | 6.3 (0.3 - 09.9) | 106 | 50.0 (25.0 - 66.9) | 304 |
| Lambda cyhalothrin and Olyset | 14.9 (9.6 - 20.6) | 17.6 (10.5 - 29.8) | 0.002 | 755 (40) | 6.8 (2.0 - 17.7) | 98 | 66.7 (42.9 - 91.6) | 232 |
| Lambda cyhalothrin and PermaNet | 20.6 (15.3 - 26.5) | 22.9 (13.5 - 38.9) | < 0.001 | 802 (40) | 6.3 (0.3 - 13.6) | 110 | 64.3 (50.0 - 80.0) | 307 |
| Lambda cyhalothrin and Icon Life | 21.6 (16.8 - 26.9) | 22.1 (13.3 - 36.7) | < 0.001 | 1055 (40) | 5.1 (1.4 - 18.9) | 114 | 62.7 (46.6 - 77.6) | 364 |

* Refers to the model estimated mean percentages as calculated in R.

[^] The term 'n' refers to total number of replicates

[§] The number of replicates (n) was the same as for *An. arabiensis*

**Controls refer to unsprayed huts in which volunteer used untreated nets

Table 6: Summary statistics table for percentage number of mosquitoes that died each night during the **second spray round**. Table showing the median percentage (and inter-quartile ranges (IQR)), model estimated mean percentages with 95% confidence intervals (for *Anopheles arabiensis*) and actual number of dead mosquitoes (total killed). The estimated mean mortalities were further corrected by Abbots formula [36].

| IRS/LLIN combinations | Mortality of <i>Anopheles arabiensis</i> | | | Mortality of <i>Culex</i> | | Mortality of <i>Mansonia</i> species | |
|---------------------------------------|--|--|-------------------------------|---------------------------|--------------|--------------------------------------|--------------|
| | Median % (IQR) | Model estimates *Mean% (95% CI) P value | Total killed (n) [^] | Median% (IQR) | Total killed | Median% (IQR) | Total killed |
| Untreated nets only** | 10.4 (04.2 - 18.1) | 0.0 (0.0 - 0.0) | 968 (90) | 3.3 (0.0 - 10.0) | 137 | 0.0 (0.0 - 27.1) | 85 |
| Olyset only | 14.8 (09.3 - 23.9) | 15.7 (12.9 - 18.7) | 1742 (90) | 2.9 (0.0 - 10.0) | 128 | 0.0 (0.0 - 42.5) | 86 |
| PermaNet only | 19.7 (11.2 - 30.1) | 19.3 (15.9 - 23.0) | 1644 (90) | 3.8 (0.0 - 13.7) | 177 | 26.1 (0.0 - 50.0) | 147 |
| Icon Life only | 16.7 (07.2 - 26.4) | 28.5 (24.8 - 32.3) | 2121 (90) | 2.3 (0.0 - 11.5) | 187 | 20.0 (0.0 - 46.6) | 198 |
| Actellic and untreated nets | 23.4 (12.9 - 36.7) | 37.3 (31.0 - 43.9) | 1599 (60) | 5.7 (2.5 - 31.8) | 272 | 21.1 (3.9 - 50.0) | 119 |
| Actellic and Olyset | 20.3 (12.4 - 31.2) | 27.9 (22.7 - 33.4) | 2171 (60) | 7.1 (3.6 - 21.0) | 291 | 31.7 (12.7 - 56.2) | 149 |
| Actellic and PermaNet | 25.0 (14.6 - 36.9) | 40.9 (34.5 - 47.4) | 2146 (60) | 9.7 (4.1 - 28.6) | 284 | 50.0 (29.4 - 97.7) | 262 |
| Actellic and Icon Life | 21.8 (11.9 - 34.2) | 43.5 (37.1 - 49.8) | 2305 (60) | 9.6 (3.8 - 33.6) | 316 | 45.0 (28.6 - 79.5) | 282 |
| DDT and untreated nets | 17.1 (08.0 - 28.3) | 19.1 (14.8 - 24.1) | 943 (60) | 3.6 (0.0 - 14.0) | 109 | 8.0 (0.0 - 38.3) | 68 |
| DDT and Olyset | 19.2 (11.6 - 28.1) | 21.0 (16.8 - 25.7) | 1201 (60) | 4.3 (0.0 - 11.1) | 124 | 22.5 (0.0 - 50.0) | 65 |
| DDT and PermaNet | 19.4 (12.6 - 34.1) | 25.3 (20.5 - 30.5) | 1171 (60) | 4.8 (0.0 - 24.3) | 150 | 33.3 (0.0 - 66.7) | 97 |
| DDT and Icon Life | 14.7 (09.7 - 24.1) | 26.6 (21.2 - 32.4) | 1255 (60) | 4.6 (0.0 - 10.6) | 151 | 1.5 (0.0 - 30.6) | 60 |
| Lambda cyhalothrin and untreated nets | 17.8 (10.4 - 28.6) | 16.3 (12.5 - 20.8) | 1431 (60) | 9.7 (4.9 - 22.5) | 197 | 21.1 (9.2 - 45.7) | 138 |
| Lambda cyhalothrin and Olyset | 14.2 (09.0 - 27.7) | 24.4 (19.7 - 29.6) | 1578 (60) | 5.5 (0.0 - 15.4) | 157 | 25.0 (0.0 - 50.0) | 136 |
| Lambda cyhalothrin and PermaNet | 19.0 (10.8 - 33.4) | 28.9 (23.6 - 34.6) | 1768 (60) | 7.7 (2.6 - 23.6) | 189 | 50.0 (8.5 - 80.0) | 264 |
| Lambda cyhalothrin and Icon Life | 18.4 (09.3 - 26.2) | 23.9 (19.0 - 29.4) | 1893 (60) | 8.0 (1.5 - 16.9) | 155 | 33.3 (16.2 - 50.0) | 210 |

* Refers to the model estimated mean percentages as calculated in R.

[^] The term 'n' refers to total number of replicates

[§] The number of replicates (n) was the same as for *An. arabiensis*

**Controls refer to unsprayed huts in which volunteer used untreated nets

Table 7: Monthly median percentage mortality of *Anopheles arabiensis* mosquitoes collected per night in experimental huts fitted with different IRS and LLIN treatments during the **first spray round (4 months) and the **second spray round** (6 months)**

| IRS/LLIN combinations | Monthly median % mortality (and IQR) during the first spray round | | | | **Corrected monthly median %mortality during the second spray round | | | | | |
|---------------------------------------|---|--------------------|--------------------|--------------------|---|---------|---------|---------|---------|---------|
| | Month 1 | Month 2 | Month 3 | Month 4 | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
| Untreated nets only | 5.4 (3.3 - 11.1) | 8.9 (7.1 - 20.6) | 8.5 (1.5 - 16.7) | 4.5 (3.0 - 08.1) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Olyset only | 8.0 (5.0 - 17.6) | 12.0 (7.5 - 17.3) | 11.6 (6.0 - 15.4) | 12.2 (8.5 - 20.8) | 21.9 | 8.0 | 3.2 | 4.3 | 3.3 | 5.9 |
| PermaNet only | 17.6 (8.7 - 23.7) | 14.3 (13.1 - 27.0) | 26.3 (19.4 - 29.5) | 20.6 (17.2 - 26.5) | 24.8 | 10.6 | 1.4 | 8.2 | 3.8 | 10.9 |
| Icon Life only | 9.8 (6.3 - 29.0) | 18.2 (09.7 - 22.5) | 19.7 (14.5 - 24.2) | 28.3 (14.3 - 34.6) | 27.1 | 8.1 | 3.0 | 4.1 | 1.6 | 4.0 |
| Actellic and untreated nets | 27.9 (22.5 - 38.5) | 14.1 (11.2 - 30.7) | 16.1 (12.3 - 19.2) | 13.1 (10.9 - 25.2) | 40.8 | 18.6 | 10.2 | 9.8 | 15.3 | 8.3 |
| Actellic and Olyset | 22.9 (15.9 - 52.3) | 17.3 (11.3 - 29.1) | 13.9 (12.1 - 19.9) | 15.2 (12.9 - 19.2) | 35.0 | 9.5 | 7.5 | 12.3 | 15.2 | 5.7 |
| Actellic and PermaNet | 29.7 (22.0 - 43.0) | 18.5 (14.4 - 40.5) | 31.1 (27.4 - 36.1) | 21.7 (16.9 - 34.2) | 44.7 | 21.7 | 13.5 | 12.6 | 8.2 | 7.0 |
| Actellic and Icon Life | 39.2 (16.0 - 50.4) | 23.2 (15.9 - 31.1) | 21.0 (13.2 - 26.7) | 15.5 (11.7 - 20.6) | 44.1 | 20.3 | 9.4 | 9.8 | 13.9 | 5.1 |
| DDT and untreated nets | 9.8 (4.7 - 20.3) | 10.0 (7.0 - 24.4) | 22.7 (11.0 - 26.9) | 18.5 (10.7 - 27.3) | 27.4 | 10.9 | 7.9 | 7.1 | 6.3 | 2.9 |
| DDT and Olyset | 9.5 (6.1 - 17.4) | 12.0 (7.7 - 16.8) | 14.0 (8.5 - 16.5) | 17.2 (9.4 - 24.4) | 17.1 | 12.0 | 10.1 | 5.0 | 12.1 | 8.0 |
| DDT and PermaNet | 17.3 (9.6 - 26.0) | 16.7 (11.0 - 21.1) | 17.0 (14.3 - 27.0) | 19.3 (11.1 - 27.0) | 27.4 | 15.6 | 9.2 | 6.3 | 15.5 | 6.4 |
| DDT and Icon Life | 10.5 (5.2 - 19.5) | 10.9 (8.6 - 16.5) | 15.2 (11.5 - 28.1) | 13.2 (9.6 - 18.2) | 29.6 | 5.7 | 3.7 | 7.0 | 5.4 | 8.9 |
| Lambda cyhalothrin and untreated nets | 16.8 (9.3 - 20.7) | 12.4 (8.3 - 20.0) | 15.5 (13.0 - 34.8) | 14.8 (11.2 - 22.6) | 20.6 | 5.4 | 3.3 | 13.0 | 7.3 | 6.2 |
| Lambda cyhalothrin and Olyset | 11.3 (8.6 - 20.2) | 11.6 (8.1 - 16.9) | 15.8 (12.0 - 21.9) | 20.4 (11.4 - 23.9) | 26.1 | 1.1 | 1.8 | 4.7 | 4.2 | 4.4 |
| Lambda cyhalothrin and PermaNet | 14.3 (10.5 - 29.1) | 20.3 (14.4 - 28.3) | 23.1 (19.0 - 25.8) | 19.5 (16.3 - 26.9) | 31.6 | 19.5 | 15.3 | 6.1 | 11.6 | 4.8 |
| Lambda cyhalothrin and Icon Life | 17.4 (11.6 - 21.9) | 17.2 (9.7 - 27.7) | 25.9 (18.6 - 32.2) | 23.8 (20.9 - 28.7) | 26.9 | 11.1 | 3.8 | 8.5 | 9.0 | 3.2 |

**** Due to excessively high mortalities in the controls, the median mortalities in the second spray round were corrected using Abbots formula [36]. The corrected inter-quartile ranges are not shown for the second spray round.**

Direct protection from mosquito bites

During the first spray round, regardless of whether IRS treatments were combined with the nets or not, less than 0.5% of all live *An. arabiensis* mosquitoes caught in any of the huts and less than 1% of the dead ones, were either fed or partly fed. Overall, all the IRS or LLINs treatments and the controls (which consisted of intact untreated mosquito nets used correctly), therefore provided greater than 99% protection from potentially infectious bites by the malaria vector, *An. arabiensis*. Similar results were obtained in the second spray round, where less than 1% of all live or dead *An. arabiensis* mosquitoes caught in any of the huts, with any of the treatments, were fed or partly fed. Thus all the treatments and the controls all provided greater than 99% protection from potentially infectious bites by the malaria vector, *An. arabiensis*. These calculations are based on the assumption that all the fed and partly fed mosquitoes had obtained their blood meals from the human volunteers sleeping inside the different experimental huts as the huts were located far from other potential sources of blood meals.

Proportions of mosquitoes caught while exiting the experimental huts versus proportions caught inside the huts

In both spray rounds, most of the mosquitoes were caught inside the exit traps as opposed to inside the experimental huts. During the first spray round, the exit trap catches accounted for at least 94.5% of all mosquitoes collected from any of the huts. The *An. arabiensis* mosquitoes found inside of the huts accounted for an average of 5% of the total catches of this species, the maximum percentage indoor catch being merely 6.3%, in the huts having actellic IRS and untreated nets. Even in the unsprayed

experimental huts having only non-insecticidal nets (i.e. the controls), 96.2% of *An. arabiensis*, 96.9% of *Culex* and 89.5% of *Mansonia* mosquitoes were caught while exiting the huts as opposed to inside the huts. Similarly, during the second, even the collections from the control huts, consisted of 98.5% of *An. arabiensis*, 97.8% of *Culex* and 97.8% of *Mansonia* mosquitoes exit trap catches, meaning that the indoor catches were in all cases less than 5%. Similarly high percentages of mosquitoes caught in treated huts were from exit traps rather than inside the huts.

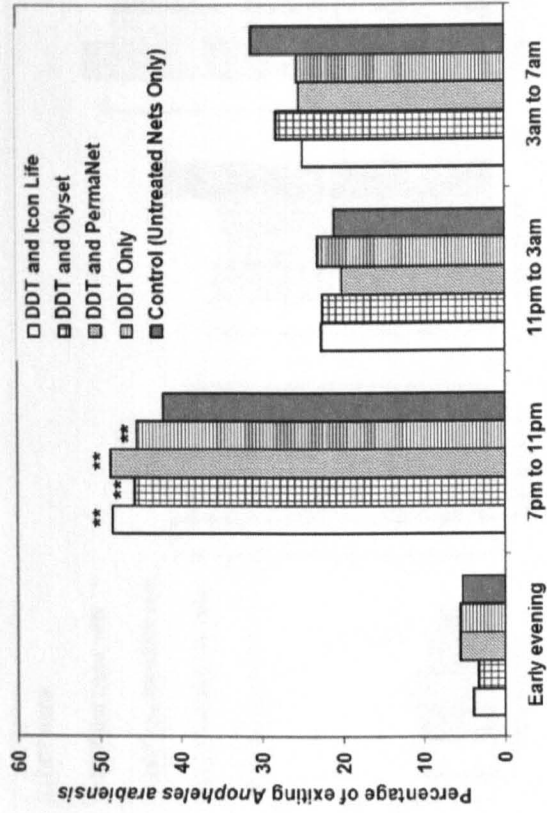
Time of the night when mosquitoes leave human occupied experimental huts

Considering only those mosquitoes that were caught exiting, the tendency to exit huts earlier was examined among the *An. arabiensis* mosquitoes caught in experimental huts with the different insecticidal treatments, relative to the controls. Figure 2 and Figure 3 show the patterns of mosquito exit, during the first and the second spray rounds, respectively. During the first spray round, the greatest percentage of exiting mosquitoes consisted of those caught between 7pm and 11pm, but this pattern shifted slightly but significantly whenever any of the insecticidal applications were used in the huts, such that this 7pm-11pm proportion was significantly increased ($P < 0.05$). The only exception was with Actellic IRS, which did not have this effect (Wald Chi Square = 1.549, $P = 0.213$). The general exit pattern however remained unchanged, meaning that most of the mosquitoes were still exiting during the same time period (Figure 2). The greatest shift towards early exit was observed in huts having actellic IRS combined with PermaNet® nets (Wald Chi Square = 65.095, $P < 0.001$), and in huts having Icon Life® nets alone (Wald Chi Square = 65.322, $P < 0.001$), both of which resulted in 53.6% of the *An. arabiensis* mosquitoes exiting in between 7pm and 11pm compared to the controls where an average of 42.9% were exiting at the same

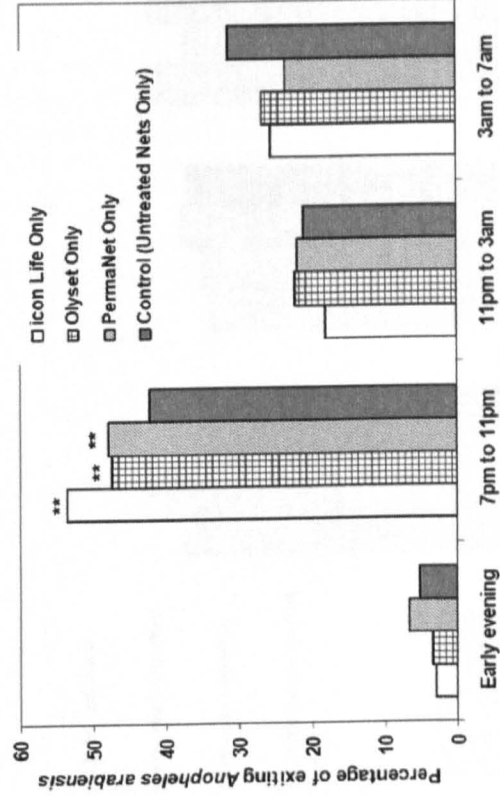
period. Many of the other treatments caused less than 10% increase in this early exit rate (Figure 2).

During the second spray round, more of the *An. arabiensis* exit from the control huts occurred at dawn. As shown in Figure 3, the greatest percentage of the exiting mosquitoes was observed to be between 3am and 7am, most likely due to seasonal shifts since round I was conducted during the dry season when temperatures are on average higher and round II during the wet season when temperatures are on average lower. However, when many of the LLINs, IRS of their combinations were introduced, this pattern shifted so that most of the mosquitoes were now exiting earlier in the night, i.e. between 7 and 11pm. When nets were introduced into unsprayed huts only PermaNet induced exophily (Wald Chi Square = 7.263, $P < 0.007$). Of the IRS treatments only actellic induced exophily (Wald Chi Square = 8.56, $P < 0.003$), although combining nets with IRS induced increased exophily with the exception DDT and Olyset® nets (Wald Chi Square = 0.044, $P = 0.834$). Similar to the first spray round, the greatest shift here was also observed in huts having actellic IRS combined with PermaNet® nets (Wald Chi Square = 44.329, $P < 0.001$), which resulted in 38% mosquitoes exiting in the period between 7pm and 11pm, compared to 29% in the exiting the controls at the same period. In both spray rounds, there were also apparent but marginal increases in rate of irritancy when the IRS and LLINs were used together relative to whenever either the LLINs or the IRS were used alone (Figures 2 and 3).

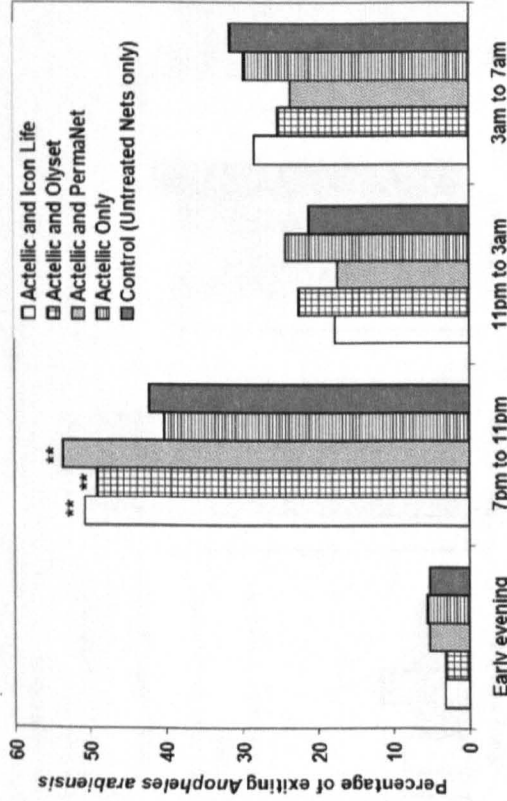
DDT huts



Unsprayed huts



Actellic huts



Lambda cyhalothrin huts

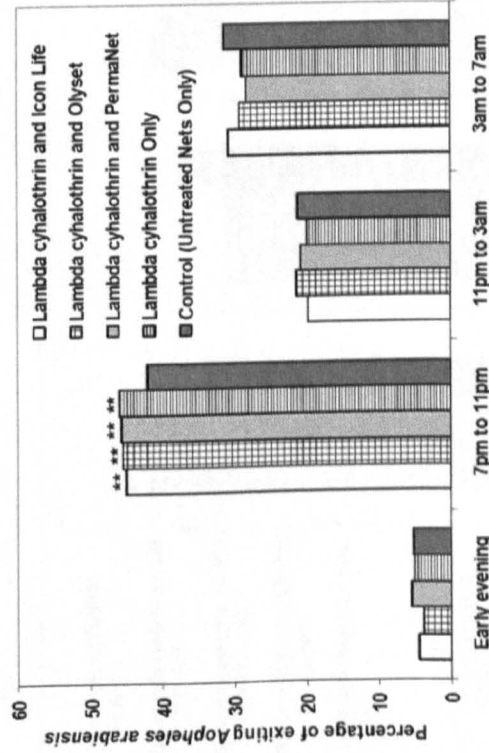


Figure 2: Effects of IRS/LLIN applications on the time when *Anopheles arabiensis* exited volunteer-occupied experimental huts during the **first spray round**. Bars marked with two stars (**) denote irritant applications that caused significantly more mosquitoes ($P < 0.05$) to exit earlier than in the controls.

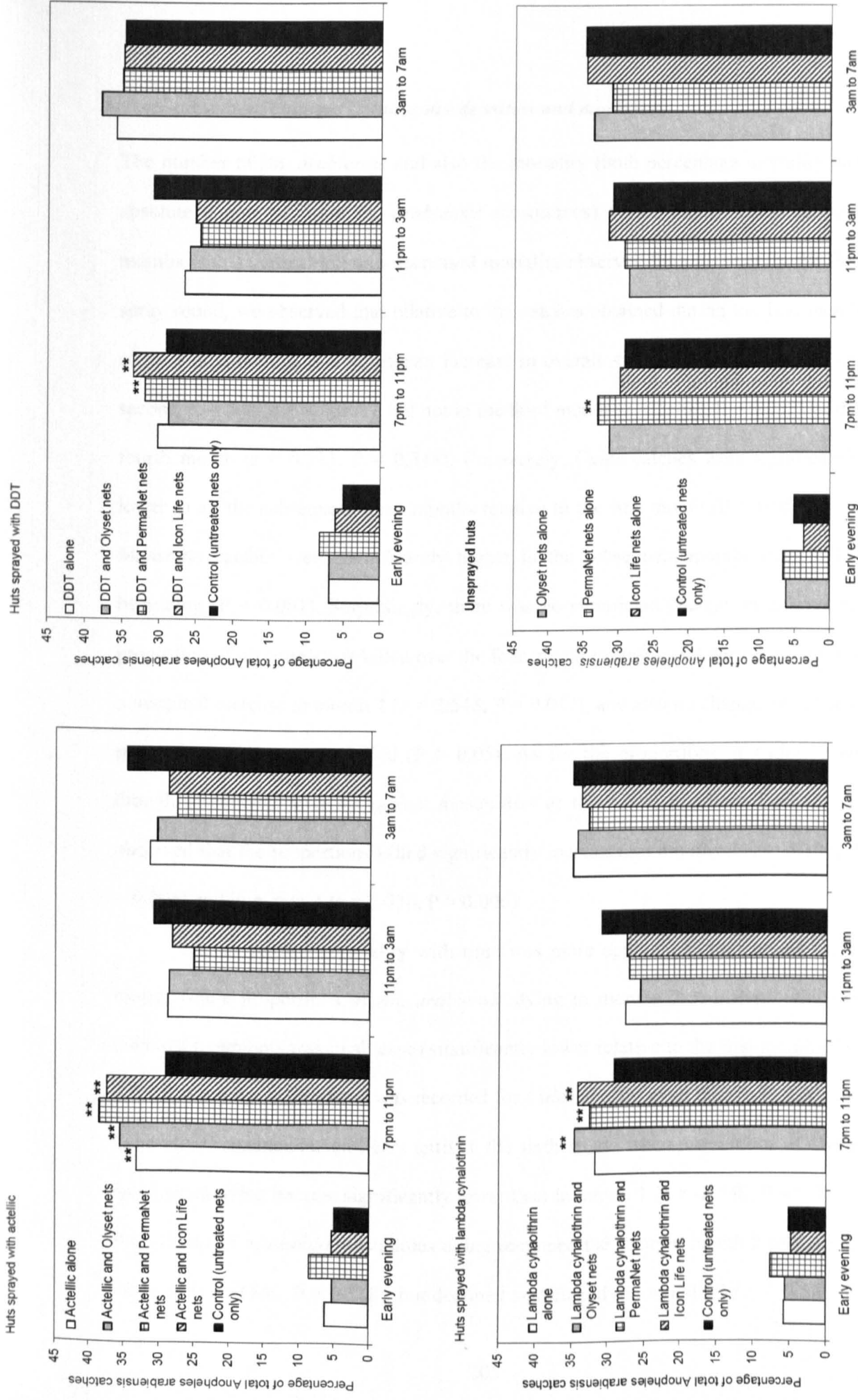


Figure 3: Effects of IRS/LLIN applications on the time when *Anopheles arabiensis* exited volunteer-occupied experimental huts during the **second spray round**. Bars marked with two stars (**) denote irritant applications that caused significantly more mosquitoes ($P < 0.05$) to exit earlier than in the controls.

Time-dependent changes in mosquito densities and mortality

The number of *An. arabiensis* and also the mortality (both percentage mortality and absolute counts of dead *An. arabiensis* mosquitoes) varied significantly between months with a general trend in decreased mortality observed through time. In the first spray round, we observed that relative to the catches obtained during the first month of the study, there was a significant increase in overall *An. arabiensis* catches in the second ($z = 5.043$, $P < 0.001$), but not in the third month ($z = 1.902$, $P = 0.057$) or the fourth month ($z = 0.131$, $P = 0.318$). Conversely, *Culex* catches were significantly lower in all the subsequent three months relative to the first month ($P < 0.05$), while *Mansonia* catches were significantly higher in the subsequent months than in the beginning ($P < 0.001$). Surprisingly, there was no significant change on the overall proportion of *An. arabiensis* killed over the four month experimental period, except for a marginal increase in month 2 ($z = 2.548$, $P = 0.012$), and also no change on relative proportions of *Mansonia* killed ($P > 0.05$). As for the proportions of *Culex*, even though insecticidal toxicity against mosquitoes of this taxon had been limited, we observed that the proportions killed significantly increased in months 3 ($z = 5.189$, $P < 0.001$) and in month 4 ($z = 2.730$, $P = 0.006$).

The decrease in mortality with time was more apparent in the second spray round, where proportions of *An. arabiensis* dying in months 2-6, in huts with the different treatments was in all cases significantly lower relative to the first month ($P < 0.001$). A similar observation was recorded for *Culex* species even though in this case the decline remained insignificant until at the sixth month when proportions of *Culex* mosquitoes dying became significantly lower than in month 1 ($z = -2.488$, $P = 0.013$). Proportions of *Mansonia* mosquitoes dying remained the same in month 2 relative to month 1 ($z = 0.646$, $P = 0.518$), but declined significantly in months 3 ($z = -2.587$, P

=0.010), month 4 ($z = -3.127$, $P = 0.002$), month 5 ($z = -3.401$, $P = 0.001$) and month 6 ($z = -2.433$, $P = 0.015$). Lastly, significant reductions were also observed on the actual numbers of mosquitoes killed in months after the start of the study. In the case of the malaria vector *An. arabiensis* a significant reduction in abundance was observed at month 4 ($z = -2.384$, $P = 0.017$), month 5 ($z = -8.863$, $P < 0.001$) and month 6 ($z = -8.954$, $P < 0.001$). Interestingly for *Culex* mosquitoes, there was no difference in actual numbers dead in month 2 relative to month 1 ($z = 1.933$, $P = 0.053$), month 4 ($z = 0.141$, $P = 0.888$) and month 5 ($z = 0.030$, $P = 0.976$), but there was a significant increase in the numbers killed in month 3 ($z = 3.526$, $P < 0.001$) and month 6 ($z = 3.880$, $P < 0.001$). No difference was observed in the number of dead *Mansonia* mosquitoes except for a slight reduction in month 2 ($z = -2.061$, $P = 0.039$).

Relative to the catches obtained during the first month of the study, there was a significant increase in overall *An. arabiensis* catches in the second ($z = 3.994$, $P < 0.001$), third month ($z = 4.578$, $P < 0.001$) and fourth month ($z = 3.368$, $P < 0.001$), and a significant decrease beginning the fifth month ($z = -2.658$, $P = 0.008$) of the study. The *Culex* mosquito catches were however significantly higher in all months relative to the first month ($P < 0.001$). On the other hand the *Mansonia* mosquito catches remained the same in the second month of the study ($z = -0.329$, $P = 0.742$), but then became significantly higher throughout the rest of the study period relative to the first month ($P < 0.001$). This fluctuation was due to the availability of breeding habitats influenced by local rainfall.

Relationship between total number of mosquitoes caught and the proportions that died

To assess whether the huts that had more mosquitoes were also the huts that had greater proportions dead, and to examine whether the huts design features such as baffled spaces were letting out mainly live mosquitoes, a statistical relationship was examined between the total catches and mortality among the catches. This analysis was conducted using only the *An. arabiensis* catches. It was observed that there was no association between these variables except for a marginally significant correlation in huts with actellic and Olyset[®] nets ($R^2 = 0.08$, $P = 0.027$).

Table 9 shows values for all the important indicators of association as observed in various huts with different treatments. If the high mosquito catches in treated huts were due to the fact that live mosquitoes were leaving and that only the killed mosquitoes were remaining, then one would expect that there is a significant relationship between these two variables, total catch and proportional mortality.

Table 12: Assessment of relationships between total numbers of *Anopheles arabiensis* caught in different huts and the level of mortality in those huts (i.e. proportion of the mosquitoes that died).

| ITN/IRS combinations used | | Indicators of relationship between mortality and total <i>Anopheles arabiensis</i> catches | | | | | |
|--------------------------------------|----------------------------------|--|-----------|------------------|------------|-------------------------------|------------------|
| | | R | R squared | Adjusted squared | R F change | Degrees of freedom Regression | P value Residual |
| Unsprayed Huts | Untreated net alone | 0.10 | 0.01 | -0.00 | 0.94 | 1 | 0.335 |
| | Olyset alone | 0.03 | 0.00 | -0.01 | 0.07 | 1 | 0.786 |
| | PermaNet alone | 0.16 | 0.03 | 0.02 | 2.51 | 1 | 0.117 |
| | Icon Life alone | 0.08 | 0.02 | -0.01 | 0.58 | 1 | 0.449 |
| Huts sprayed with actellic | Actellic alone | 0.22 | 0.05 | 0.03 | 2.96 | 1 | 0.091 |
| | Actellic and Olyset | 0.28 | 0.08 | 0.07 | 5.14 | 1 | 0.027 |
| | Actellic and PermaNet | 0.02 | 0.00 | -0.02 | 0.01 | 1 | 0.907 |
| | Actellic and Icon Life | 0.14 | 0.02 | 0.00 | 1.19 | 1 | 0.280 |
| Huts sprayed with DDT | DDT alone | 0.22 | 0.05 | 0.03 | 3.14 | 1 | 0.081 |
| | DDT and Olyset | 0.09 | 0.01 | -0.01 | 0.44 | 1 | 0.512 |
| | DDT and PermaNet | 0.17 | 0.03 | 0.01 | 1.69 | 1 | 0.199 |
| | DDT and Icon Life | 0.07 | 0.01 | -0.01 | 0.30 | 1 | 0.588 |
| Huts sprayed with lambda cyhalothrin | Lambda cyhalothrin alone | 0.20 | 0.04 | 0.02 | 2.41 | 1 | 0.126 |
| | Lambda cyhalothrin and Olyset | 0.07 | 0.01 | -0.01 | 0.32 | 1 | 0.572 |
| | Lambda cyhalothrin and PermaNet | 0.02 | 0.00 | -0.02 | 0.03 | 1 | 0.868 |
| | Lambda cyhalothrin and Icon Life | 0.06 | 0.00 | -0.01 | 0.18 | 1 | 0.671 |

Discussion

All the accrued achievements in malaria control notwithstanding, many malaria experts now believe that successful control and eventual elimination of the disease in many parts of Africa will require additional new tools, and an optimal integration of the existing methods such as LLINs and IRS [23, 37-40]. Given that conclusive proof remains one of the key requirements for decision-making regarding LLIN/IRS combinations, the purpose of this current study was to contribute direct empirical evidence for or against this strategy, by conducting actual field studies in a malaria endemic area. All treatments were assessed relative to a control consisting of untreated bed nets, such that volunteers who slept inside the experimental huts were always afforded the basic level of personal protection from potentially harmful mosquito bites. The study was conducted in two spray rounds, the second round including a set of incremental improvements over the first spray round. The study enabled direct comparisons of different treatments on the basis of a variety of attributes namely: a) the number of mosquitoes entering different huts, b) proportions and actual numbers of mosquitoes that died after exposure to the various treatments, c) the times when mosquitoes exited the huts with various insecticidal applications, d) the proportions of mosquitoes prevented from feeding upon the volunteers and e) the proportions of mosquitoes caught exiting the huts as opposed to remaining indoors.

Given the research methodology applied and the results of this study, there are at least two ways to focus on our most important question, which is whether LLIN/IRS combinations can prevent exposure to malaria transmission more than either LLINs alone or IRS alone. First, where IRS is already in place, addition of LLINs would be clearly beneficial by enhancing direct protection against bites (i.e. feeding inhibition) and to a small extent by killing additional malaria mosquitoes in

the house. Mosquito deterrence on the other hand is obviously not an important protective property of LLINs. Based on the results of this study, it is clear that all tested bed net types, including the untreated nets, when used correctly function mainly by preventing mosquitoes from feeding upon hut occupants, but that there is no deterrence of malaria mosquitoes except with PermaNet® nets that have a limited deterrent effect. Relative to any of the IRS treatments when used alone, none of the net types resulted in a decrease in number of mosquitoes entering the huts, suggesting that any additional benefits from LLINs would not possibly be due to improved deterrence at household level, but that it would be due to direct prevention of bites at an individual level and direct toxicity of the LLINs to malaria mosquitoes providing community level protection. Moreover, results from both spray rounds show an increase in mosquito counts in huts having pirimiphos methyl IRS versus and/or Icon Life® nets. Though we are not yet able to explain this apparent attractiveness of the two treatments, future research should examine this possibility and determine whether the treatments have an even greater potential as candidates for lure and kill strategies.

The second way to look at the question of additional protection is to consider situations where LLINs are the pre-existing interventions, in which case, the results from this study are mixed. It is clear especially from the first spray round that whereas IRS using DDT would provide additional household level protection by deterring mosquitoes from entering huts thus reducing man-vector contact, no additional benefit can be expected from DDT due to toxicity. Moreover, IRS treatments are not known to prevent vector feeding on their own, meaning that other than the minor deterrent effects of DDT, additional protection from IRS treatments would mainly be the result of increased toxicity. Of the tested IRS compounds, only actellic significantly increased the proportional mortality relative to what is achievable with LLINs used

alone. Lambda cyhalothrin exhibited only a limited degree of toxicity while DDT did not appreciably induce additional mortality relative to LLINs used alone. It seems therefore that where people already use any of the LLINs, additional improvements by IRS can be obtained only where the chemical of choice is either actellic (as used in this study), or some other approved compound with similar properties. This suggestion matches the current proposals by both WHO and also a number of experts who are concerned about overexposing mosquitoes to insecticides of the same class, which would increase the likelihood of insecticide resistance [17, 21, 41]. Given actellic is an organophosphate, combining it with any of the LLINs, all of which are currently pyrethroid based, would therefore not only provide additional household protection, but the insecticide combination would also potentially mitigate against the rise and progression of resistance alleles among vector populations [17, 41]. This kind of strategy is already being widely suggested for consideration as a means of preserving effectiveness of existing vector control tools [40, 41]. Indeed, on a very positive note, hut trials recently conducted in Benin, west Africa confirmed that combinations of LLINs with chlorfenapyr, a pyrole insecticide can have enhanced impact by killing greater proportions of mosquitoes bearing insecticide resistance genes, relative to LLINs used on their own [21].

The level of mortality observed in our experimental hut study is lower than observed in many previous studies, the results of which were presented in the supplementary online materials accompanying the review article by Okumu and Moore [17]. Other than the differences in experimental hut designs [25], one possible explanation for the low mortalities in our experiments is that all the nets used in this study were intact (un-holed) nets; and that even the control huts had intact untreated nets rather than no nets at all. This means mosquitoes were restricted from feeding

upon the hut occupants, and were more likely to exit the huts and continue host seeking. Indeed, this study also shows that less than 1% of the collected mosquitoes were blood fed (fully or partly). As a result, there were not many mosquitoes resting in these huts after feeding, which would otherwise have translated to higher post-feeding mortality. Previous studies have shown that IRS treatments kill mostly blood fed mosquitoes [21, 42], mainly because these are the mosquitoes that rest for long periods on the treated surfaces. It is thus possible that our experimental set up, with intact nets as controls, may have to a certain degree, misrepresented real life situations where poor care of LLINs leads to damage after just a few months of use, and therefore led to an underestimation of toxicity.

However, it is also likely that the low mosquito mortalities in this study are linked to the fact that most of the collected mosquitoes (> 95% in all cases) were actually caught while exiting the huts. Besides, the data on *time of exit* suggests that this egress was occurring soon after the mosquitoes entered the experimental huts (Figures 2 and 3). This observation coupled with the fact that we conducted our collections multiple times a night (i.e. every four hours), suggests that the mosquitoes visited the huts normally but exited soon afterwards, most likely because they had not been successful in finding any blood meals in the huts. Clearly, the mosquitoes were not spending sufficient time in the huts to receive a fatal exposure to insecticides. While it is natural that unfed mosquitoes would continue their host seeking activity [43], what is also very important to consider is the indication that these mosquitoes, or at least the local *An. arabiensis* populations, tend to give up on any individual hosts whom they find protected with nets, and therefore readily fly out of huts where users have intact nets or use insecticide [44]. This is not surprising as it matches the behaviour of *An. arabiensis*, which is of course known to be a fairly opportunistic

feeder [45] and could also explain why *An. arabiensis* populations have been reduced to a lesser extent by ITNs than *An. gambiae* s.s. [27, 46].

There are two inferences from these observations that are important in terms of public health benefits. First, the mosquitoes that fail to feed are less likely to rest on the inside hut walls and are therefore less likely to be killed by any IRS insecticides, a very likely explanation for our observation of comparatively low mortality rates in this trial than in most previous studies, where holed nets were deliberately used to allow mosquitoes to feed on sleeping volunteers and therefore rest while digesting their blood meal [21]. Secondly, constantly deterring mosquitoes to the extent that they give up on host seeking within any household would inevitably result in a desirable blanket protection at community level, if used at sufficiently high coverage [47]. Therefore, despite the mosquito behaviour and the possibility that reduced toxicity may substantially reduce communal benefits, the personal protection that nets provide when combined with either deterrent or toxic IRS at household level remains significantly protective, and can be readily extrapolated to entire communities by increasing the intervention coverage across the human population[17], also as detailed in Chapter VIII.

In addition to computing the proportional mortality among mosquitoes that entered different experimental huts, we also examined and directly compared the actual numbers of mosquitoes killed in huts that had the different treatments, relative to the controls. The main reason for this was to extrapolate directly what the contributions of these insecticidal applications would be in terms of community level protection minus the effect of the physical barrier that a net provides. By killing a larger number of mosquitoes than the controls each night, any insecticidal application would have a considerably higher community level effect [48]. This study has shown

that huts sprayed with actellic would result in the greatest community level effect, but that significant benefits are also achievable in huts with lambda cyhalothrin when supplemented with either Icon Life® or PermaNet® nets. Other than the direct protection from mosquito bites as observed from the low blood feeding rates in houses with LLINs, it should be recognized that houses fitted with LLIN or IRS treatments actually act like large mosquito killing stations; where mosquitoes are lured into the houses and then killed. In this regard therefore, where intact nets are available to users, highly effective contact toxicants such as actellic based IRS and Icon Life® nets or combinations consisting of these interventions, which let in large numbers of malaria mosquitoes and kill a large proportion of those mosquitoes, would provide a greater community level impacts than interventions that let in and kill fewer mosquitoes due to deterrent or irritant modes of action while still protecting the individuals in that household. This point of view has been expressed by malariologists for many years, including by world renowned experts, Prof. Chris Curtis and Dr. Abraham Mnzaza [49], who suggested over a decade previously that non-irritant insecticides should be favoured for IRS over the pyrethroids because the latter make insects leave the site of treatment (i.e. excito-repellents) thus reducing mosquito mortalities.

Perhaps the most important reason why people use nets is to prevent mosquito bites. For most users, this generally includes nuisance mosquitoes such as many of the *Culex* and *Mansonia* mosquito species, which may also transmit a number of neglected tropical infections [50]. This study has clearly demonstrated that at household level, all nets, including the untreated nets, can prevent blood feeding by more than 99%. These high protection levels were achieved in all huts regardless of whether they had been sprayed with any of the IRS compounds or not. Obviously it

would be illogical to expect higher feeding success rates in the controls since the controls actually also had intact nets, albeit untreated ones. On the contrary, this result can be interpreted to mean that even untreated bed nets if consistently used and kept intact, will provide high levels of protection from mosquito bites, as has been shown previously elsewhere [27, 51, 52], and can themselves significantly improve the benefits achievable from IRS, relative to IRS alone. The study also clearly shows that with regard to prevention of mosquito bites at household level, no added advantage should be expected from adding IRS where most people already use LLINs or untreated nets. This bold view is however somewhat simplistic as it assumes the very unlikely scenarios that: 1) all net owners would properly and consistently use the nets, and 2) the users stay under their nets all the time when they are in their houses and 3) that the nets remain intact all the time.

Though this observation on feeding inhibition was made only at household level, one would argue that in communities where most residents, say 80-90% use these nets, host seeking mosquitoes would be deterred consistently, eventually creating a blanket community effect where these mosquitoes die of starvation or predation as they search for alternative hosts, likely to be wild animals, cows, chicken etc. Indeed many previous studies have shown that interventions that have significant deterrent effects notably DDT would lead to near extinction of the main vectors especially where these vectors feed almost exclusively on humans [47, 49, 53]. Therefore, another very important inference from this study is the potential of a high coverage of consistently used and intact untreated intact nets in providing necessary public health benefits and possibly even eliminating the need for insecticide treatment. In foresight, we would like to suggest that the new LLIN technologies, which reduce the probability that nets become holed, could be utilised to create long

lasting untreated nets (LLUN), which would then be applicable in rotations with current LLINs, or in combinations with current IRS treatments, as a way of insecticide resistance management.

Lastly, we also analysed, based on our exit trap catches, the tendency of *An. arabiensis* mosquitoes to exit huts earlier than normal (Figures 2 and 3). The intention was to examine if any of the insecticidal applications actually had an irritant effect on the mosquitoes, which would lead to more mosquitoes getting out of the house; potentially improving household level protection, especially where such exits take place before mosquitoes feed [17], but also potentially undermining communal benefits of LLINs, by forcing the mosquitoes out before they take up lethal insecticide doses [22]. Data from the first spray round suggest that most of the applications tended to increase early exit, but also that even where the greatest of this shift occurred, the general pattern of exit remained same as in the controls, such that the proportion of all exits remained greatest between 7pm and 11pm. Results from the second spray round however showed that whereas in the controls, the greatest percentage of exiting mosquitoes consisted of those caught exiting between 3am and 7am each (Figure 2), i.e. at dawn, this pattern tended to shift slightly so that after introduction of the insecticidal applications, most of the mosquitoes were now exiting earlier in the night, between 7 and 11pm. Considering the need to protect not only the intervention users but also non-users, it is perhaps important to realize that the period between 7pm and 11pm coincides with the time when people are still going about their business outdoors and that mosquito species, particularly *An. arabiensis*, might exploit the situation and become more dangerous to people outdoors at this time of the night.

Compared to IRS alone, the additional benefit achievable from forced early exit can however be expected to be minimal. For example, in actellic and DDT sprayed huts, we observed only marginal increments in the rate of irritancy whenever the LLINs were added, relative to whenever the IRS applications were used alone. We are not aware of any previous study suggesting that excessive early exits would have any eventual public health benefit, and further studies will be needed to clarify this aspect. On one hand, it is logical to assume that by increasing the early exit rate, especially where the exiting mosquitoes do so without having been successful at feeding upon hut occupants, household level protection would be proportionately increased, so that where most houses are protected in the same way, a desirable level of community protection can be achieved. On the other hand however, there remains the possibility of antagonism at household level, where highly irritant IRS would cause the mosquitoes to without having contacted treated surfaces e.g. LLINs indoors [22, 49], or without having picked up large enough doses of the insecticides to kill them. In such a case, insecticides that cause early exit would be disadvantageous. Therefore, the question of whether impacts of this early exit can be large enough to warrant investments to reduce it through improved formulation should be investigated further.

Whereas it is possible to generalise observations made in this study for several other studies as well, it is important to note that the local mosquito populations in this area have undergone significant changes in composition over the past years. Whereas *An. gambiae* s.s. used to be the most common malaria vector in the area, data from this current study and from several recent collections consistently suggest that more than 95% of malaria vectors in the area, are now *An. arabiensis* [27]. Thus we would be more inclined to generalise these results only to other areas where the dominant

vector species is either *An. arabiensis* or has similar behavioural and physiological characteristics as the populations in this study site and not to all areas. Also, it should be noted that insecticide susceptibility tests, standard WHO bioassays on treated walls and on nets as well as molecular examination of mosquitoes from this area do not point at any known insecticide resistant mechanisms, but instead give indications that susceptibility to commonly used public health pyrethroids may be weakening [54]. A more descriptive analysis of the bio-efficacy and residual effects of the treatments is presented in Chapter V.

A related but more immediate concern from the results presented here relate to the low toxicity and deterrence achievable using Olyset® nets, which are currently the commonest in Tanzania. Compared to the other two LLINs that we tested, this brand was the least toxic and had minimal deterrence. All the Olyset® nets, we tested were obtained directly through the local supply chains, meaning that these results are very likely to be representative of the efficacy of this brand of nets as is currently being used in Tanzania.

Conclusion

This study involved evaluation of LLINs and IRS treatments in the best possible conditions, where they are used consistently and properly the whole night. We conclude that: 1) there are minimal additional protective benefits to be gained from adding IRS with DDT or lambda cyhalothrin into houses where people already use existing LLINs consistently, 2) given the available range of insecticides for malaria control, combining pyrethroid based LLINs with IRS would be most effective if the IRS of choice were a highly toxic and non irritant chemical such as actellic, a combination which would also provide an additional advantage of being suitable for

resistance management 3) intact untreated nets, by merely preventing mosquito bites, can constitute an effective complementary intervention to be used alongside IRS, where LLINs are not readily available and 4) where resources are limited, the focus should be that everyone in a malaria risk area uses an LLIN consistently, instead of trying to combine LLINs with IRS. Nevertheless, we also recognize that in situations where it is not possible to provide everyone with LLINs or where the LLINs cannot be maintained in an intact state, and in epidemic situations, IRS with highly toxic insecticides should be added to provide the necessary communal protection by killing excess malaria mosquitoes. Thus the current practice by WHO should be continued in the sense that IRS should be promoted in communities where malaria epidemic risk is high and also in areas where there are low rates of net-use.

Acknowledgements

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Author contributions

FO, JM, BS and SJM designed and conducted the field and laboratory experiments, EM, GL, supervised the field experiments, FO, LL and SJM conducted the statistical analysis. FO, under supervision of SM wrote the manuscript.

Conflicts of interest

None

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Bio-efficacy and persistence of insecticides used for indoor residual spraying and long lasting insecticide nets: results from laboratory and field evaluations against the malaria vector, *Anopheles arabiensis* in south-eastern Tanzania*

Abstract

Background: We assessed the bio-efficacy and residual activity of insecticides used for indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs), against laboratory-reared and wild populations of the malaria vector, *Anopheles arabiensis* in south-eastern Tanzania. This was a complementary study conducted alongside an experimental hut study aimed at assessing synergies and redundancies in household level protection, when IRS is combined with LLINs.

Methods: WHO bioassays were performed using cones and wire balls to assess residual activity of insecticides in LLINs, and those sprayed on mud walls and palm-thatched ceilings of experimental huts. WHO-susceptibility tests were also performed using diagnostic concentrations of candidate insecticides, against wild mosquitoes collected in the study area. Lastly, molecular analysis was performed to detect knock-down resistance genes associated with resistance against DDT and pyrethroids.

Results: Whereas all candidate IRS formulations (DDT wettable powder, lambda cyhalothrin capsule suspension and pirimiphos-methyl (actellic) emulsified concentrate), were highly effective during the first month after spraying (killing $\geq 85\%$ of mosquitoes

* Adapted from: Okumu F., Mbeyela E, Ligamba G., Moore J., Chipwaza B. and Moore J: *Bio-efficacy and persistence of insecticides used for indoor residual spraying and long lasting insecticide nets in an area of weakening susceptibility among the malaria vector species, *Anopheles arabiensis*, in south-eastern Tanzania, Manuscript in Preparation*

exposed in cone bioassays) these treatments rapidly decayed losing most activity within 1-3 months. The tested LLINs (Olyset®, PermaNet® and Icon Life®) also lost insecticidal efficacy, in some cases by > 50% in six months, although they were not washed in this period. Malaria vectors in this study area were fully susceptible to DDT and no knock-down resistance gene mutations were detected. However, weakening susceptibility to lambda cyhalothrin and permethrin was observed, necessitating vigilance against emerging pyrethroid resistance.

Conclusions: Existing pyrethroid-based LLINs remain the most efficacious intervention against malaria vectors in this area. Given the rapid decay of insecticidal activity on the mud surface, and possibility that mosquitoes might not rest long enough on treated surfaces to pick up lethal doses, IRS when used alone is minimally appropriate for vector control in this scenario. If these results are interpreted in the context of the more general objective, to determine if there are any added advantages of combining LLINs with IRS, there is clear justification for adding LLINs where IRS is the only existing intervention, especially to provide continued protection when the IRS decays. There is however, no evidence to support introduction of IRS into houses where LLINs are already being used. The potential for resistance emerging in the area should be carefully monitored.

Introduction

Decisions to use indoor residual spraying (IRS), insecticide treated nets (ITNs) or the two methods together for malaria vector control in any given area are usually based on existing epidemiological conditions, operational requirements and the expected protective efficacy of the interventions [1]. Of these factors, protective efficacy is itself a function of the behaviour of local mosquito populations [2] and also susceptibility of these vectors to those insecticides used for the ITNs or IRS [3].

In an earlier study, we evaluated three different insecticides approved by WHO for use in IRS campaigns (lambda cyhalothrin, actellic and DDT) and also three types of LLINs (Olyset[®], PermaNet[®] and Icon Life[®]), the first two of which are already widely used in malaria endemic areas. The aim of that study, which was conducted using experimental huts, was to determine if there can be any additional benefit of combining LLINs with IRS as opposed to using either of the methods alone (Chapter IV). Here, we report on a complementary study conducted in parallel, to assess the bio-efficacy and residual activity of insecticides used in the LLINs and IRS that were under evaluation.

Materials and methods

Study area and mosquitoes

The study was conducted in Lupiro village (8.385°S and 36.670°E) in Ulanga District, south-eastern Tanzania (see Okumu *et al.*, 2010 [4] for detailed description of the study area). The mosquitoes used for this study were either wild female *Anopheles arabiensis* mosquitoes caught inside experimental huts constructed in the study village, or they were from a new mosquito colony that was established using offspring

from blood fed *An. arabiensis* mosquitoes collected from local human houses the same study area.

LLINs and IRS compounds

Four net types (three LLINs and one non-insecticidal net) and three IRS insecticides of different classes (one organochloride, one synthetic pyrethroid, and one organophosphate) were tested. The candidate LLINs were: Olyset® nets (a permethrin-impregnated net manufactured by A-Z, Tanzania), PermaNet 2.0® nets (a deltamethrin-coated net, manufactured by Vastergaard, Switzerland) and Icon Life® nets (a deltamethrin- impregnated net, manufactured by Syngenta, Switzerland). Similarly, the candidate IRS compounds were those tested in the earlier LLIN/IRS study and included 2g/m² DDT wettable powder (AVIMA, South Africa), 0.02g/m² lambda-cyhalothrin capsule suspension, (produced by Syngenta, Switzerland) and an emulsified concentrate of 2g/m² pirimiphos-methyl emulsifiable concentrate, also known as actellic (Syngenta, Switzerland). These IRS insecticides had been sprayed on walls and ceilings of selected experimental huts using standard WHOPES procedures [5]. The walls of these experimental huts were plastered using local mud, which locals use for house building because of its high clay content, while the ceilings were made of palm woven mats locally known as *Mikeka*.

Assessment of residual activity of the IRS insecticides and LLINs

Based on WHO guidelines for testing mosquito adulticides [6], bioassays were conducted *insitu* to examine residual activity of the insecticides in the bed nets, and on the hut walls and ceilings, at specific intervals during the period of the LLIN/IRS

combination study. To do this, blood fed *Anopheles gambiae* complex mosquitoes were collected from local houses in the same study village where the LLIN/IRS study had been taking place. The mosquitoes were kept in separate water filled vials and left to lay eggs, after which adults was identified by polymerase chain reaction (PCR), to distinguish between *An. arabiensis* and *An. gambiae s.s* [7], the two sibling species of *An. gambiae* complex found in the study area. Using the eggs obtained from *An. arabiensis* (which constituted >99% of all the field samples), an insectary colony was established and maintained in a semi-field system inside a screen house at the Ifakara Health Institute [8], to provide mosquitoes for bioassays. The larvae here were regularly fed on ground fish food and adult mosquitoes maintained on 10% sugar solution, at temperatures of 28 - 29°C and 70-80% relative humidity.

Residual efficacy of bed nets: Cone bioassays and wire ball tests [6] were conducted on newly unbundled nets, and thereafter once every month, for the six months period during which the LLIN/IRS study was conducted. Differences between cone and wire ball assays are as follows: in the cone assays, mosquitoes are exposed by enclosing in close proximity to test surfaces using plastic cones. This method can be used on any flat surfaces including nets surfaces, walls and ceilings. The wire ball method on the other hand consists of two intersecting circular frames of wire, each measuring 15cm diameter, around which test nets are wrapped to form a netting ball. This method can be used on nets but not on walls or ceilings [6].

Batches of 5 mosquitoes (for cone tests) or 11 mosquitoes (for wire ball tests) were exposed for 3 minutes on each of the 5 sides of the nets as described in the WHO guidelines [6]. The mosquitoes were all 2–5 days old nulliparous females. After exposure, the number of mosquitoes knocked down within 60 minutes was recorded.

All mosquitoes were then provided with 10% glucose solution inside a holding room where mean indoor temperatures were $29.1^{\circ}\text{C} \pm 3.0^{\circ}\text{C}$ during the day and $26.7^{\circ}\text{C} \pm 2.3^{\circ}\text{C}$ at night, while mean relative humidity was $70.6\% \pm 17.9\%$ during the day and $75.7\% \pm 13.7\%$ at night. Mosquitoes were monitored for 24 hours, after which the numbers of dead and surviving mosquitoes were recorded. Dead mosquitoes were defined as mosquitoes not standing on their legs at the usual 45 degrees angle, and incapable of any movement when disturbed. Controls, consisting of non-insecticidal mosquito nets, were included alongside each of the assays, and up to 4 different nets of each type were tested per month.

Residual efficacy of IRS: Batches of 10 nulliparous females (2–5 days old) from the screen house colony were introduced into the WHO cones and exposed for 30 minutes on each of the four walls of each hut and also on two randomly selected positions on the ceilings of each of the sprayed experimental huts. The mosquitoes were monitored for 24 hours as above and mortality recorded. The first of these bioassays on walls and ceilings were done in freshly sprayed experimental huts (i.e. two days after the spraying). Repeat bioassays were conducted once every month for the six-month duration of the LLIN/IRS combination study. Controls, which consisted of unsprayed hut walls and ceilings, were included in each of these assays.

A similar set of bioassays was performed on separate wooden panels (1m^2), lined with either mud or *Mikeka*, to simulate the walls and ceilings of the experimental huts respectively. The panels had been treated with insecticides the same way as the experimental huts, by attaching them onto the inside surfaces of the door shutters, so that they were sprayed at the same time as the huts were being sprayed. These panels were kept inside the same experimental huts so as to ensure they

remained under the same environmental conditions as the sprayed walls and ceilings, for as long as the LLIN/IRS combination experiment lasted. However, unlike in the experimental hut bioassays, which were conducted either on vertical surfaces (in the case of sprayed walls) or downward facing horizontal surfaces (in case of ceilings), all assays on the wooden panels were conducted with the panels kept on a flat horizontal surface. There were two mud panels and two *Mikeka* panels sprayed with each of the test IRS compounds. Ten mosquitoes were exposed on four different spots per panel, so that a total of 80 mosquitoes were tested per treatment per surface per month. Controls used here consisted of unsprayed *Mikeka* and mud panels.

Susceptibility of local malaria vectors to insecticides used for IRS and LLINs

Adult mosquitoes were collected using exit traps attached to experimental huts, inside which adult male volunteers slept under non-insecticidal nets. The experimental huts and also the traps used for this purpose have previously been described elsewhere [9]. For this specific purpose, we used those huts that had not previously been sprayed with any insecticide, during the LLIN/IRS combination study (Chapter IV). Mosquitoes collected from the huts were provided with 10% sugar solution and maintained under ambient shade conditions in a holding room at our study site, for up to five hours before being used. Each morning after mosquito collection, mosquitoes were identified morphologically to select *An. gambiae* s.l females, which were then subjected to standard WHO insecticide-susceptibility bioassays [10]. Recent molecular analyses of *Anopheles gambiae* mosquitoes from this study village have consistently shown that > 97 % are *An. arabiensis* [4, 11].

The insecticide-susceptibility bioassays [10] were performed by exposing the selected nulliparous female mosquitoes 2-4 days old to filter papers impregnated with

diagnostic concentrations of deltamethrin (0.05%), permethrin (i.e. 0.75%), lambda cyhalothrin, (i.e. 0.05%), dieldrin (i.e. 0.4%) and DDT (i.e. 4%). The assays were performed at near-room temperature conditions ($25 \pm 2^{\circ}\text{C}$), making sure that the exposure tubes are always held vertically. All the insecticide-impregnated papers as well as papers used as controls, and all the insecticide-testing tubes and mouth aspirators were supplied by the Vector Control Research Unit, Universiti Sains Malaysia.

In each test 21 to 25 mosquitoes were exposed to the insecticide-impregnated papers for up to 60 minutes in tubes lined with the respective insecticide impregnated papers (Figure 1). During exposure the number of mosquitoes knocked down in each tube was recorded after 10, 15, 20, 30, 40, 50 and 60 minutes. After the 60 minutes exposure, mosquitoes were transferred into clean holding tubes and kept for 24 hours in the holding room, during which time they were provided with 10% sugar solution. Where no knock-down was observed within the initial 60 minutes of exposure, the mosquitoes were transferred from the insecticidal test tubes to the clean holding tubes and observed after an additional 20 minutes. Mortality was monitored and recorded after the 24 hour holding period. We tested a maximum of 125 mosquitoes per insecticide, which was equivalent to 5 replicates of 25 mosquitoes per test. Since we were also unable to collect enough *An. arabiensis* females to conduct all the assays at the same time, the replicate tests were conducted in consecutive days, making sure that we had one control each day. Figure 1 shows insecticide susceptibility tests being conducted at a field station in the study village.

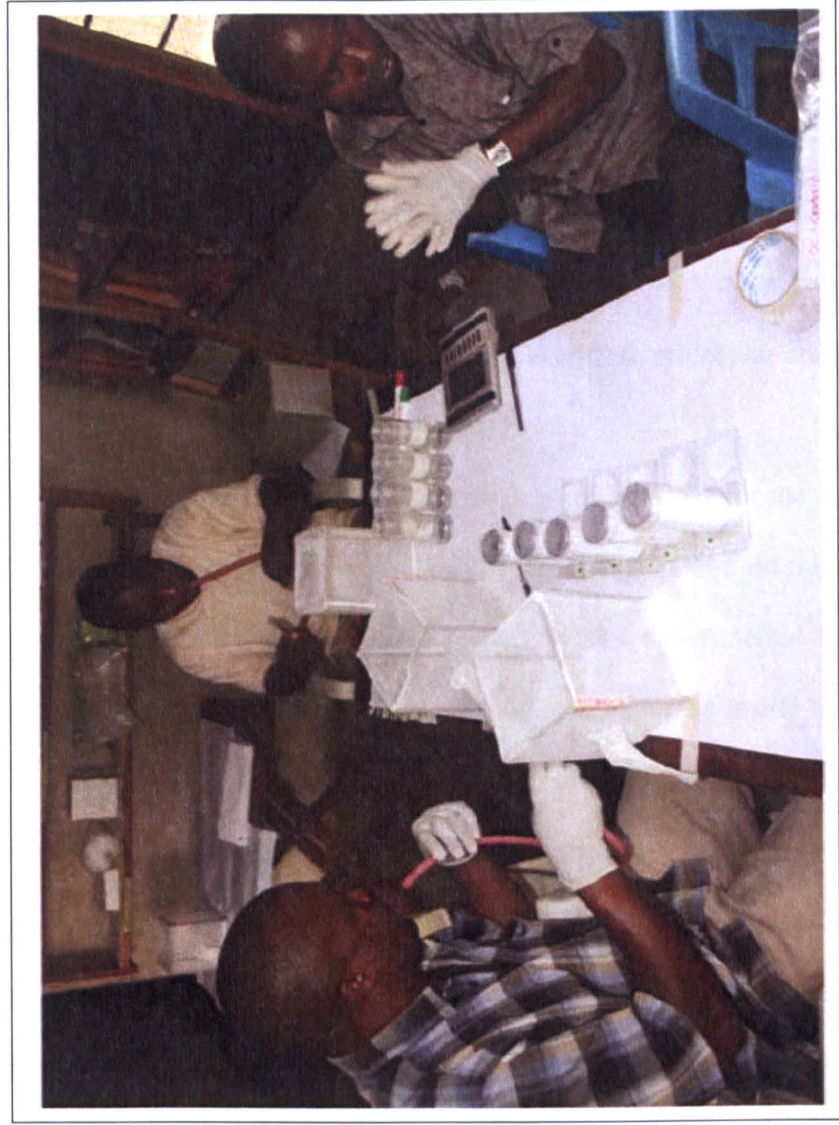


Figure 1: Technicians performing insecticide susceptibility tests

Molecular analysis of frequency of knock down resistance (kdr)-gene mutation in the local mosquito population

Given that that we initially observed low percentage mortalities even among mosquitoes caught in experimental huts with WHO approved insecticidal interventions [12], it was reasonable to suspect that insecticide resistance was present. Given that most of the interventions that we tested were pyrethroid based (Olyset[®], PermaNet[®] and Icon Life[®] nets, and lambda cyhalothrin for IRS), and because we also tested DDT for IRS, one of our major concerns was possibility that any such resistance would be associated with presence of knock-down resistance (*kdr*) alleles [13] among local mosquito populations. Therefore molecular analysis was performed with the aim of detecting *kdr* presence.

We included four different groups of mosquitoes for the *kdr* analysis, namely:

1) wild *An. arabiensis* mosquitoes collected using CDC-light traps from local houses in the same study village where our LLIN/IRS experimental hut study was being conducted: 2) wild *An. arabiensis* mosquitoes collected inside the experimental huts used in the LLIN/IRS study, 3) mosquitoes originating from the *An. arabiensis* colony that we established using mosquitoes originally collected from the same study village, as described above and 4) mosquitoes which had survived the WHO bioassays performed on the insecticide-sprayed walls, sprayed ceilings and the nets, also as described above. Courtesy of Dr. Raphael N'guessan of Centre de Recherche Entomologique, Cotonou, Benin , positive controls were obtained from an area in Benin, where *kdr* allele frequency has been consistently shown to be > 95% in recent years [14]. The detection of *kdr* using PCR was performed at Ifakara Health Institute, Tanzania. We adapted a protocol originally developed by Martinez-Torres *et al.* [15]

for detection of both the L1014S *kdr* allele (mutation commonly found in East Africa [16, 17]) and L1014F *kdr* allele (mutation commonly found in West Africa [15, 18]).

Data analysis

The mortality of mosquitoes in the different bioassays was calculated as a proportion of the total number exposed to each chemical. Abbotts formula was used to correct the mortality in all tests where the control mortality was higher than 10%. In the susceptibility tests, the percentage knock-down was also calculated for each of the time periods when the mosquitoes were observed.

Molecular distinction of *An. gambiae* complex sibling species

A sub-sample of all the female *An. gambiae s.l* mosquitoes collected in the experimental huts and in the local houses for the bioassays, was examined using multiplex PCR, using the protocol originally developed by Scott *et al.* [7] to determine proportions of *An. gambiae s.s.* and *An arabiensis*. All the wild mosquitoes subjected to *kdr* examination were also subjected to the PCR for species identification.

Protection of participants and ethical approval

Human participants in this study included the volunteers who slept in the experimental huts during the time when adult mosquitoes were being collected for use in the insecticide susceptibility tests. Participation of volunteers in these experiments was voluntary, even though all participants received nightly wages. After full

explanation of purpose and requirements of the studies, written informed consent was sought from each volunteer prior to the start of all experiments.

While inside the experimental huts, the volunteers slept under intact bed nets as a basic protection against mosquito bites. They were also provided with long sleeved, hooded jackets to provide additional protection from bites, whenever the volunteers stepped outside the nets to collect mosquitoes from the exit traps attached to the huts. In addition, the volunteers were provided with access to diagnosis for malaria parasites using rapid diagnostic test kits, and treatment with the current first-line malaria drug (artemether-lumefantrine) in case they had malaria. Ethical approval for this work was granted by the Institutional Review Board of the Ifakara Health Institute (IHRDC/IRB/No.A019), the Tanzania National Institute of Medical Research (NIMR/HQ/R.8aNo1.W710) and the London School of Hygiene and Tropical Medicine (Ethics Clearance No. 5552).

Results

Residual activity of candidate insecticidal applications on malaria transmitting mosquitoes: results of the monthly bioassays

Figure 2-4 show residual activity of insecticides sprayed on mud walls and ceilings of experimental huts, and also activity of the LLINs on *An. arabiensis* mosquitoes over a period of six months. Additional data including total numbers of mosquitoes exposed per test is provided in supplementary tables S1-S4. During the first month of spraying, 100% of mosquitoes exposed to *Mikeka* ceilings sprayed with either actellic or lambda cyhalothrin died, whereas only 85% were killed by DDT. On the mud walls

sprayed with the same chemicals, we observed 100%, 90.0% and 97.5% mortality respectively during the first month (Figure 2).

Activity of the IRS declined significantly within just two months, so that by the third month, actellic killed only 42.5% of mosquitoes exposed to sprayed ceilings and only 55.0% of those exposed to treated walls. Lambda cyhalothrin on the other hand killed only 46.3% on ceilings and 52.5% on walls. By month 6, actellic had nearly entirely decayed, killing only 7.5% of *An. arabiensis* exposed to sprayed ceilings and on 27.5% of those exposed to sprayed walls. By this time, lambda cyhalothrin was now killing only 30.0% on ceilings and 27.5% on walls. The decay of DDT on either of the surfaces was however relatively much slower, and by the sixth month, it was still killing 42.5% of mosquitoes exposed to sprayed ceilings, and 36.3% of those exposed to sprayed walls (Figure 2).

The additional set of data obtained from bioassays on sprayed mud and mukeka panels depict a similar insecticide decay pattern (Figure 4), except that the *mikeka* panels remained effective for much longer than the *mikeka* ceilings. Nevertheless, these panel assays also showed that by the sixth month, most of the insecticidal activity had vanished from both mud and *Mikeka* surfaces sprayed with any of the candidate insecticides (Figure 3).

Results of the bioassays conducted on LLINs are shown in Figure 4. While all the net types generally performed better (i.e. killed more mosquitoes) on wire frame assays than on the cone assays, it was surprising that their activity rapidly deteriorated by the second month of use relative to new nets. For example, Olyset® nets killed only 69.1% of *An. arabiensis* mosquitoes exposed in the wire ball assays during the second month and only 26.0% of those exposed in the cone assays at the same time (Figure 4). Only PermaNet® nets retained mosquitocidal efficacy of 80% by the sixth month

of net use (killing 92.7% on wire ball tests and 84% on cone assays). All the LLINs however retained very high knock-down rates (> 90% in wire ball tests and >80% in cone tests) on the exposed mosquitoes, except Olyset® nets whose knock-down activity reduced to 72.7% on wire ball tests and 62% on cone tests by the sixth month.

Susceptibility of local *An. arabiensis* females to commonly used insecticides

Table 1 below shows the susceptibility status of *An. arabiensis* mosquitoes in the study area to the candidate insecticides. Of all the insecticides tested, 100% susceptibility was observed only for DDT. In tests on permethrin, lambda cyhalothrin and deltamethrin, we observed signs of insecticide tolerance, with susceptibilities within WHO-set range of 80%-97%, at which resistance should be suspected [10]. However, both DDT (4%) and permethrin (0.75%) elicited very high knock-down rates after 60mins of exposure, i.e. 95.2% and 99.2% respectively, while lambda cyhalothrin (0.05%) elicited only 74% knock-down and deltamethrin elicited only 85.9% knock-down after the same period of time. The lowest knock-down rates were observed with 0.4% dieldrin, which after 60 minutes had knocked down only 2.5%. We continued to monitor these mosquitoes for 80 minutes as stipulated in the WHO guidelines [10] but the knock-down rate remained very low, i.e. 26.5%.

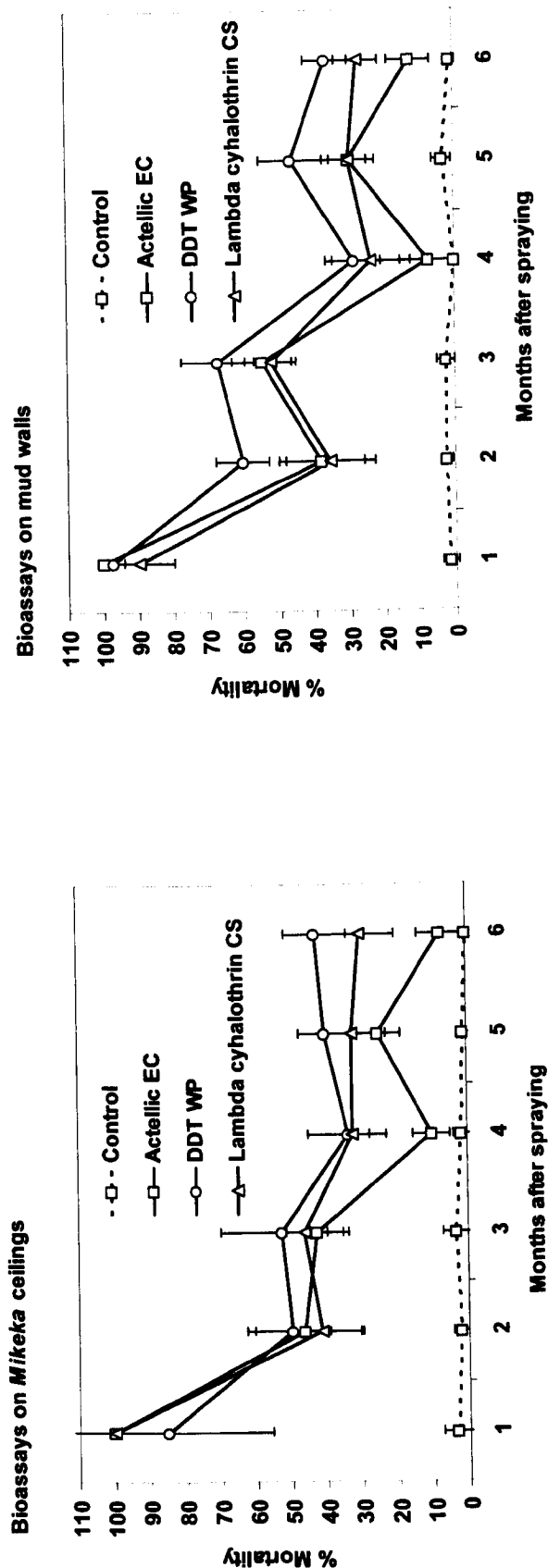


Fig. 2: Results of monthly bioassays showing residual activity of various IRS compounds sprayed on *Mikeka* ceilings and mud walls of experimental huts.

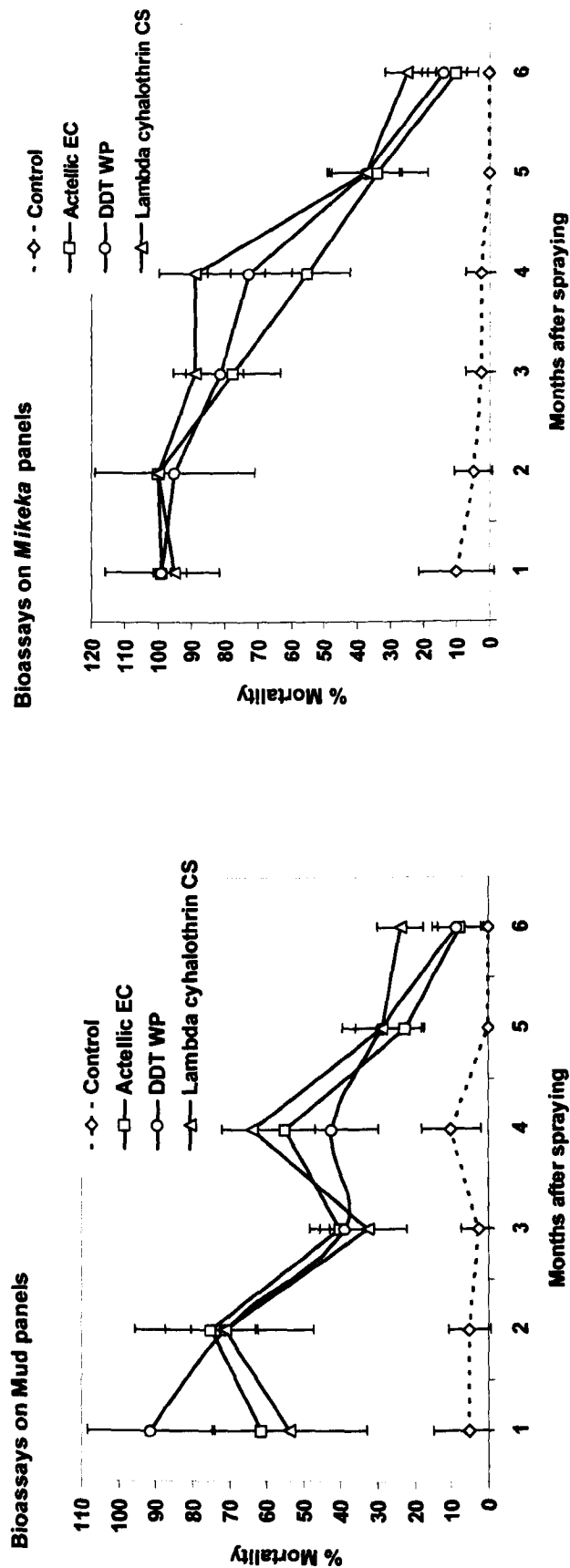


Fig. 3: Results of monthly bioassays showing residual activity of various IRS compounds sprayed on panels (1 sq metre each) lined with *Mikeka* (*Mikeka* panels) or mud (mud panels).

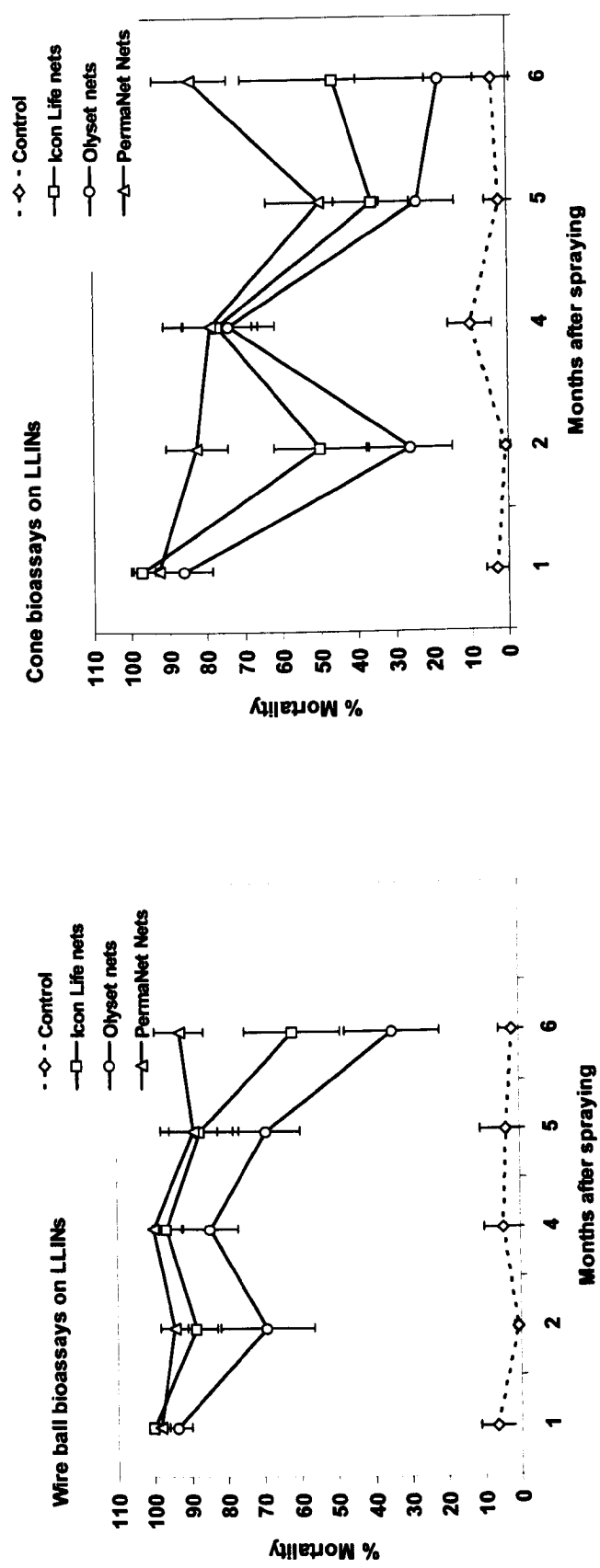


Fig.4: Results of monthly bioassays showing residual activity of various LLINs when tested using either the standard WHO cone assays or the wire ball method (two intersecting wire cycles, each measuring 15cm diameter, around which the test net is wrapped to form a netting ball). The control here consisted of a non-insecticidal mosquito net. No assays were conducted on the third month due to lack of mosquitoes.

Frequency of knock-down resistance genes among local An. arabiensis females

We analysed a total of 141 *An. arabiensis* females obtained from the colony that had been established using wild caught females from the study area. Among these mosquitoes, there were 122 successful amplifications in the PCR for detection of *kdr*, all of which were *kdr*-negative (100%). Though, these mosquitoes included those that had survived the standard bioassays on the hut walls and nets, they were all negative for *kdr* alleles. Of the 522 mosquitoes obtained from our experimental huts during the LLIN/IRS combination study described earlier (Chapter IV), we obtained 383 successful amplifications in both the *kdr* detection PCR and species identification PCR. Again, all of these were determined to be *An. arabiensis* and all were *kdr*-negative (100%). Finally, we also analysed 43 mosquitoes collected directly from local houses in the study area, using CDC light traps set near bed nets. In this case only 15 showed successful amplifications in the PCR for both *kdr* detection and species identification, all of which were identified as *An. arabiensis* and also as being 100% *kdr*-negative.

Table 1: Results of the insecticide susceptibility tests conducted on wild *Anopheles gambiae s.l* mosquitoes

| | Knock Down (KD) | | | | | | | | | | Mortality | |
|---------------------------|-------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|----------------------|-----------|-------------------------|
| | Total No. exposed | KD 10mins | KD 15mins | KD 20mins | KD 30mins | KD 40mins | KD 50mins | KD 60mins | %KD 60mins | Total No. Dead | % Dead | Susceptibility Class |
| | | | | | | | | | | | | |
| Control | 123 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0.8 | 1.0 | 0.8 | - |
| DDT, 4% | 124 | 0 | 0 | 7 | 44 | 94 | 114 | 118 | 95.2 | 124.0 | 100.0 | *** |
| Permethrin, 0.75% | 125 | 0 | 8 | 20 | 71 | 95 | 122 | 124 | 99.2 | 119.0 | 95.2 | ** |
| Lambda cyhalothrin, 0.05% | 123 | 0 | 0 | 2 | 23 | 60 | 81 | 92 | 74.8 | 111.0 | 90.2 | ** |
| Deltamethrin, 0.05% | 96 | 0 | 5 | 16 | 37 | 60 | 74 | 79 | 85.9 | 92 | 95.8 | ** |
| Diieldrin, 0.4% | 124 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2.4 | 120.0 | 96.8 | ** |

Susceptibility classification

*** The percentage mortality value indicates 100% susceptibility

** The percentage mortality value indicates insecticide tolerance and possibility of resistance that needs to be confirmed

Discussion

This study was designed to complement a separate study, which was conducted to evaluate common LLINs and IRS insecticides when used alone or when combined (Chapter IV). The results therefore provide important clues on the bio-efficacy of public health insecticides currently being used for malaria vector control, particularly how they are likely to perform in an area where malaria vectors remain susceptible, albeit with clear signs of that this susceptibility is declining. In Tanzania, ITN use has been increasing significantly in the past decade [19]. High coverage with ITNs has been actively supported through a voucher scheme, which was scaled up to nationwide reach in 2008 [20]. Catch up campaigns with permethrin treated LLINs (Olyset® nets) targeting children under fives began in 2008 [21], and the government with support of partners, mainly the US President's Malaria Initiative (PMI), has been actively implementing IRS campaigns in a number of epidemic-prone districts [22]. The potential for insecticide resistance to emerge against common IRS/LLIN insecticides must therefore be very carefully monitored.

Insecticide susceptibility is usually classified based on the proportions of mosquitoes that die when exposed to diagnostic concentrations of test chemicals as follows: 98-100% mortality indicates susceptibility, 80-97% mortality indicates signs of resistance that need to be confirmed and less than 80% mortality indicates that there is insecticide resistance [10]. In a recent nationwide study in Tanzania, where insecticide resistance was assessed in several districts, it was shown that susceptibility of mosquito populations to lambda cyhalothrin, deltamethrin and permethrin had started to diminish in most of the sentinel districts in the country, including Kilombero district, which neighbours Ulanga district where this current study was conducted [23]. In that study, standard WHO insecticide susceptibility tests on *An.*

gambiae s.l from Kilombero district, showed 93.9% mortality after exposure to 0.05% lambda cyhalothrin, 96% mortality after exposure to 0.75% permethrin and 90.3% mortality after exposure to 0.05% deltamethrin [23]. Results from this current study (Table 1), depict a closely similar pattern, two years later, i.e. full susceptibility to the diagnostic concentrations of DDT, and reduced susceptibility to lambda cyhalothrin (mortality = 90.2%), permethrin (mortality = 95.2%) and deltamethrin (mortality = 95.8%). While the resistance limits in this area have not yet reached a state where vector control interventions such as pyrethroid based LLINs and IRS with DDT would be considered ineffective, this current study also indicates the declining susceptibility of malaria mosquitoes to the common vector control insecticides, and therefore also supports the need for constant monitoring.

The good news however was that both the bioassays and the molecular analysis conducted to detect *kdr* alleles, confirmed absence of target site resistance to pyrethroids and DDT, which is one of the mechanisms linked to genetic mutations in the *para*-sodium channels in several insects [24]. Pyrethroid-DDT cross-resistance currently presents, what is perhaps the greatest challenge to insecticide based malaria interventions in Africa [25, 26]. Therefore, insecticide susceptibility surveys have now become standard pre-requisites as sources of baseline data on insecticide susceptibility status, to support the large scale LLINs and IRS campaigns in Africa [26, 27]. Two different *kdr* mutations have been found in the African malaria vector *Anopheles gambiae* s.s, including one in West Africa, which is caused by a leucine to phenylalanine substitution (L1014F) [15, 18] in the genetic sequence coding for the sodium channels, and a different mutation in East Africa, caused by leucine to serine substitution at the same amino acid position (L1014S) [16, 17]. Though the *kdr*-

detection protocol used here could detect either of the two mutations [15], we found a 100% *kdr*-negative rate in all the samples tested.

Based on percentage mortalities observed after a maximum of 30 minutes contact between mosquitoes and sprayed surfaces, this study shows that activity of the tested IRS compounds can decline significantly within the first few months after spraying, and could in some cases be considered ineffective earlier than the time when they would normally be due for re-spraying [12]. According to recommendations made by WHO [12], DDT should be re-sprayed after every 6 months, lambda cyhalothrin every 3 to 6 months and pirimiphos methyl (actellic), every 2 to 3 months [12]. As an example, we found that actellic EC, which according to our LLIN/IRS combinations study was also the most toxic to mosquitoes (Chapter IV), caused merely 42.5% mortality on ceilings and only 55.0% on walls by the 3rd month after spraying. One recent independent study also showed that this formulation, remains effective against *An. gambiae* s.l for up to 3 months, matching the existing recommendations by WHO [28].

If we consider the more practical situation where malaria control programs can feasibly afford to do only two spray rounds per year, it becomes apparent that all the other tested IRS compounds in their existing formulations would be minimally appropriate for use in this study area or in areas with similar vector populations and where people use similar construction materials for walls and ceilings. In this study, we observed that after 6 months, actellic EC was now killing only 7.5% and 27.5% of *An. arabiensis* on ceilings and walls respectively, a near complete decay. DDT WP on the other hand was killing only 42.5% on mud walls and 36.3% on ceilings, while lambda cyhalothrin CS killed only 30.0% and 27.5% respectively, six months after spraying (Figure 2). Given that actellic and lambda cyhalothrin are clearly very highly

toxic to malaria vectors when tested in experimental huts, improving their residual activity so that they can be used for longer periods, for example by using different formulations, should be emphasized in future developments. Indeed there are already some efforts to develop new formulations of these chemicals, notably actellic, that can would ensure slower release of the active ingredient and longer periods of effectiveness [29, 30].

In addition to enabling the assessment of residual activity, the wall and ceiling bioassays also highlighted how differences in treatment surface substrates can affect insecticidal efficacy. That is to say, efficacy of active ingredients on mosquitoes is modulated by type of substrate onto which the compound is applied [31]. In this study, two of the IRS insecticides, actellic EC or lambda cyhalothrin CS, killed 100% of mosquitoes exposed to the *Mikeka* ceilings, while DDT WP sprayed on *Mikeka* ceilings killed a modest 85% in the first month. However, on the mud walls sprayed with the same chemicals, we observed 100%, 90.0% and 97.5% mortality respectively in the same period. It seems therefore that, whereas lambda cyhalothrin CS, performed better on ceilings than on mud surfaces, the DDT formulation was clearly better when used on mud walls than when used on *Mikeka* ceilings, from which the water-based wettable powder would more easily have flaked off. Similar arguments have been put forth by a number of authors [31-35], and it is thought that such differences are associated with differences in adsorptive properties of the substrates. For instance, mud surfaces can be highly porous and adsorptive to insecticides, and substrates containing alkaline substances may degrade the candidate insecticide faster than substrates without alkaline contents [31, 36] In one study where pyrethroids were tested on different substrates, it was found that porous surfaces such as mud can show variability in insecticidal activity, presumably due to absorption of the insecticides,

while less porous surfaces such as wood would result in higher insecticidal activity for long periods due to lower rates of insecticide absorption [34]. More recently, Etang *et al.*, [31], also observed variations of insecticide residual bio-efficacy on different types of wall surfaces in Cameroon and therefore suggested that local construction materials should be considered when determining lengths of spray cycles.

Given the results that we have obtained from the bioassays on nets, it is clear that the two methods used here, i.e. the plastic cone and wire ball method [6], can give different outcomes, and therefore a more careful interpretation is required. The LLINs generally killed more mosquitoes in the wire ball assays than in the cone assays. According to the current LLIN testing guidelines [6], there are two possible alternatives to the WHO cones, which can also be used to assess residual efficacy of insecticidal nets, namely: 1) the use of WHO test tubes (cylinders) lined on the inside with the test nets, and 2) the wire-ball test as used in this study. It is however also suggested that further calibration against the WHO cones is required before the alternative methods can be widely used in testing and evaluation of insecticide for treatment of mosquito nets, an explanation which also suggests an expectation that the two test methods would give different results.

One may argue that since the wire ball offers no alternative resting sites (unlike in the cone assays, where mosquitoes can occasionally rest on the cotton plug used to seal the insertion hole on top of the cone, and therefore fail to make adequate contact with the test surfaces), mosquitoes are more likely to be killed in the balls than in the cones. Furthermore, if the active ingredient has irritant properties, which prevent mosquitoes from resting on treated surfaces for extended periods of time, it is possible that exposed mosquitoes would tend to frequently move from point to point making multiple contacts with the treated surfaces, and therefore leading to greater

exposure and higher percentage mortality. In this study however, we did not directly observe any mosquitoes avoiding tarsal contact with the netting material during the cone bioassays; neither did we observe many mosquitoes landing on the cotton wool that was used to plug the plastic cones, which would have indicated a significant role of irritancy [37, 38]. We believe therefore that the reason more mosquitoes died in wire ball assays than the cone assays was the greater total surface area of LLINs and consequently the greater overall quantities of insecticide that these insects were exposed to in the wire balls relative to the cones.

On the same note, these findings from the LLIN bioassays were somewhat unexpected, given our expectation that the LLINs should retain their insecticidal activity for at least 3 years and 20 washes [39]. The tests described here depict a very rapid loss of the mosquitocidal activity of the candidate LLINs; even in the wire ball tests. Whereas these products are usually made to last at least 3 years [39], with some such as the Olyset® nets designed to have up to 5 years of effective life [40], our tests show that insecticidal activity significantly actually declines significantly within the first few months. For example, Olyset® nets killed only 69.1% of *An. arabiensis* mosquitoes exposed in the wire ball assays and only 26.0% of those exposed in the cone assays by the second month of use. Only the PermaNet® nets retained a killing efficacy of 80% by the sixth month of net use (killing 92.7% on wire ball tests and 84% on cone assays). Despite this rapid decline, it is equally important to note that in this study, we also observed that all candidate LLINs retained high knock-down rates (>90% in wire ball tests and >80% in cone tests) on the exposed mosquitoes, except Olyset® nets whose knock-down activity was reduced to 72.7% on wire ball tests and 62% on cone tests by the sixth month. This particular observation presents a slightly complicated scenario in the sense that on one hand, the high knock-down rates may be

a sign that there can still be significant personal protection achievable in houses where these interventions are used, but on the other hand, the reduced knock-down rates occurring after only six months in some test nets such as the Olyset[®] nets can be considered as a reinforcement of the likelihood that pyrethroids are nonetheless beginning to lose their insecticidal potency in this area. The latter explanation is reinforced by the data from our insecticide susceptibility tests, also conducted under this study (Table 1), which showed that lambda cyhalothrin impregnated papers caused 99.2% knockdown after 60 minutes exposure, while DDT and permethrin caused only 95.2% and 74.8% knock-down respectively.

One other important aspect to consider in relation to the above is the fact that in this study the nets were not washed, at any time during the course of the study, but were instead only dusted occasionally to remove dust. The lack of washing could explain the observation that LLINs such as Olyset[®] nets, which are known to possess regenerative properties (normally activated after lengthy periods of use, after washing or after exposure to heat [40, 41]), exhibited a decline in activity during this study. Nevertheless, it is reasonable to be concerned about quality of nets that get marketed as being effective for many years. Evidence from this current study may suggest the likelihood that after just one year of use, the only effect of the net that would be left is the physical barrier effect where nets work simply to prevent mosquitoes from feeding upon the net occupants, unless the nets are regenerated through washing or exposure to sunlight, suggesting minimal difference between treated and untreated nets. Indeed, in the LLIN/IRS study (Chapter IV), we have also determined that intact non-insecticidal nets equally prevent mosquitoes from blood feeding upon net users, just as intact insecticidal nets.

Given the results from our susceptibility tests, which were conducted on wild mosquitoes, the absence of *kdr* mutation in both laboratory samples and field samples tested, and also the fact that mosquitoes used for testing residual activity of IRS and LLINs had been colonised for at least six months without any selection pressure from insecticide exposure, it is reasonable to believe that the colony did not harbour any of the insecticide resistance mechanisms that would hinder efficacy of these insecticides. It is also reasonable to believe that the colony mosquitoes were 100% susceptible to both DDT and the pyrethroids tested here.

In our earlier LLIN/IRS study, we did not observe any percentage mortalities greater than 50% with any of the insecticidal applications, even during the first month after the start of the experiments. Based on the results of this complementary study (notably the 100% mortality observed in the first month bio-assays on actellic and lambda cyhalothrin treated surfaces (Figures 2-3), the 98.2% and 100% mortality in first month wire ball assays on Icon Life and PermaNet nets respectively (Figure 4), and also the 100% susceptibility to DDT impregnated filter papers (Table 1)), we now believe that insusceptibility to any of the IRS insecticides or the LLINs is clearly unlikely to be the reason that percentage mortalities in the LLIN/IRS study were that low. Instead it appears that the actual behaviour of vectors inside our experimental huts was the major cause [2, 38]. Given that most of the mosquitoes that we captured in the LLIN/IRS trial were unfed mosquitoes caught exiting the huts, and also since we emptied out exit traps every 4 hours ensuring that exiting mosquitoes were not unnecessarily retained near treated surfaces, it is very likely that the reason we observed low percentage mortalities with the same insecticidal applications tested here, was that mosquitoes were not making adequately long contacts with the treated surfaces, and were not receiving toxic doses of insecticide. Instead the mosquitoes

were exiting the huts soon after entry and without taking blood meals, as all volunteers in the huts were covered with nets. Thus the generally low mortalities observed in that trial (Chapter IV).

If the results of this study are interpreted in the context of our general objective which was to determine if there are any added advantages of combining LLINs with IRS, relative to using each individual application separately, then this study provides very clear evidence to support the need to add LLINs where IRS is the only existing intervention. Given that most of the IRS candidate insecticides decay so quickly, and since in practice it can be difficult to regularly re-spray houses at the frequencies stipulated by WHO [12], addition of LLINs in such houses would provide not only an additional reduction in mosquito biting rates indoors, but it would also add the temporal overlap necessary to protect house occupants during the period after which the IRS is no longer efficacious. On the other hand the mortality assessments in this study present no justification for introducing IRS into houses where LLINs are already being used. As in our previous publication [1], we suggest that there may be no critical need for IRS campaigns to be launched where there is already wide coverage and correct use of LLINs, except in situations where there are epidemics and where the nets are likely to be old or torn (as is common with ordinary hand treated ITNs). However, even in such cases, the IRS treatments should preferably be those that 1) significantly deter malaria mosquitoes from entering houses, 2) are of a different insecticide class than the LLINs and 3) are implemented consistently at very high coverage throughout the communities [1].

Conclusion

We conclude from this study that the insecticidal efficacy of all the three IRS compounds, DDT WP, lambda cyhalothrin CS and actellic EC, decay very quickly within the first few months after spraying, necessitating that LLINs are used in the same households where these IRS compounds are sprayed, so as to provide the necessary protection even after IRS activity is significantly reduced. The LLINs also gradually lose their insecticidal efficacy with time, in some cases by up to 50% or more within just six months but can continue to directly protect users from mosquito bites as long as they are intact. Campaigns that highlight the need for regular net regeneration as part of correct net use have an important role in ensuring optimal malaria control. Moreover, though the malaria mosquitoes in this study area are still fully susceptible to DDT and despite the absence of knock-down resistance genes among the vector populations, there are signs of weakening susceptibility to pyrethroids, which necessitate vigilance against possibility of widespread insecticide resistance arising in this study area in the near future, especially since insecticide treated net coverage in the area is already extremely high, reaching over 90% in 2008 [42]. These findings support our earlier recommendations that: 1) where houses are already sprayed with any of the 3 tested IRS compounds, addition of LLINs would provide significant benefits by directly providing additional protection against mosquito bites and by ensuring that the people remain protected even after the IRS activity has decayed, and 2) where residents use intact LLINs, addition of IRS may not necessarily provide any significant additional benefits.

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Conflicts of Interest

None

Additional files

Table S1: results of monthly bioassays showing residual activity of various IRS compounds sprayed on hut walls and ceilings.

| Insecticide | Bioassays on <i>Mikeka</i> ceilings | | | | Bioassays on mud walls | | |
|------------------------|-------------------------------------|------------------------|-------------|----------------------|------------------------|-------------|----------------------|
| | Months | No. mosquitoes exposed | Number dead | Percentage mortality | No. mosquitoes exposed | Number dead | Percentage mortality |
| Control | 1 | 60 | 2 | 3.3 | 120 | 2 | 1.7 |
| | 2 | 240 | 5 | 2.1 | 240 | 6 | 2.5 |
| | 3 | 120 | 4 | 3.3 | 120 | 3 | 2.5 |
| | 4 | 120 | 2 | 1.7 | 120 | 0 | 0.0 |
| | 5 | 120 | 1 | 0.8 | 120 | 4 | 3.3 |
| | 6 | 120 | 0 | 0.0 | 120 | 1 | 0.8 |
| Actellic EC | 1 | 40 | 40 | 100.0 | 80 | 80 | 100.0 |
| | 2 | 160 | 74 | 46.3 | 160 | 60 | 37.5 |
| | 3 | 80 | 34 | 42.5 | 80 | 44 | 55.0 |
| | 4 | 80 | 8 | 10.0 | 80 | 6 | 7.5 |
| | 5 | 80 | 20 | 25.0 | 80 | 24 | 30.0 |
| | 6 | 80 | 6 | 7.5 | 80 | 10 | 12.5 |
| DDT WP | 1 | 40 | 34 | 85.0 | 80 | 78 | 97.5 |
| | 2 | 159 | 79 | 49.7 | 160 | 97 | 60.6 |
| | 3 | 80 | 42 | 52.5 | 80 | 54 | 67.5 |
| | 4 | 80 | 27 | 33.8 | 80 | 23 | 28.8 |
| | 5 | 80 | 32 | 40.0 | 80 | 37 | 46.3 |
| | 6 | 80 | 34 | 42.5 | 80 | 29 | 36.3 |
| Lambda cyhalothrin, CS | 1 | 40 | 40 | 100.0 | 80 | 72 | 90.0 |
| | 2 | 160 | 66 | 41.3 | 160 | 57 | 35.6 |
| | 3 | 80 | 37 | 46.3 | 80 | 42 | 52.5 |
| | 4 | 80 | 26 | 32.5 | 80 | 19 | 23.8 |
| | 5 | 80 | 26 | 32.5 | 80 | 24 | 30.0 |
| | 6 | 80 | 24 | 30.0 | 80 | 22 | 27.5 |

Table S2: results of monthly bioassays showing residual activity of various IRS compounds sprayed on separate panels made of mud or *Mikeka*.

| Insecticide used | Months | Bioassays on <i>Mikeka</i> panels | | | Bioassays on mud panels | | |
|------------------------|--------|-----------------------------------|-------------|----------------------|-------------------------|-------------|----------------------|
| | | No. mosquitoes exposed | Number dead | Percentage mortality | No. mosquitoes exposed | Number dead | Percentage mortality |
| Control | 1 | 40 | 4 | 10.0 | 40 | 2 | 5.0 |
| | 2 | 40 | 2 | 5.0 | 40 | 2 | 5.0 |
| | 3 | 40 | 1 | 2.5 | 40 | 1 | 2.5 |
| | 4 | 40 | 1 | 2.5 | 40 | 4 | 10.0 |
| | 5 | 40 | 0 | 0.0 | 40 | 0 | 0.0 |
| | 6 | 40 | 0 | 0.0 | 40 | 0 | 0.0 |
| Actellic EC | 1 | 80 | 80 | 100.0 | 80 | 60 | 75.0 |
| | 2 | 80 | 79 | 98.8 | 80 | 49 | 61.3 |
| | 3 | 80 | 62 | 77.5 | 80 | 32 | 40.0 |
| | 4 | 80 | 44 | 55.0 | 80 | 44 | 55.0 |
| | 5 | 80 | 27 | 33.8 | 80 | 18 | 22.5 |
| | 6 | 80 | 8 | 10.0 | 80 | 6 | 7.5 |
| DDT WP | 1 | 80 | 79 | 98.8 | 80 | 73 | 91.3 |
| | 2 | 80 | 76 | 95.0 | 80 | 57 | 71.3 |
| | 3 | 80 | 65 | 81.3 | 80 | 31 | 38.8 |
| | 4 | 80 | 58 | 72.5 | 80 | 34 | 42.5 |
| | 5 | 80 | 30 | 37.5 | 80 | 23 | 28.8 |
| | 6 | 80 | 11 | 13.8 | 80 | 6 | 7.5 |
| Lambda cyhalothrin, CS | 1 | 80 | 76 | 95.0 | 80 | 43 | 53.8 |
| | 2 | 80 | 80 | 100.0 | 80 | 57 | 71.3 |
| | 3 | 80 | 71 | 88.8 | 80 | 26 | 32.5 |
| | 4 | 80 | 71 | 88.8 | 80 | 51 | 63.8 |
| | 5 | 80 | 30 | 37.5 | 80 | 23 | 28.8 |
| | 6 | 80 | 20 | 25.0 | 80 | 19 | 23.8 |

Table S3: results of monthly bioassays showing residual activity of various LLINs*.

| Months | Bioassays performed using wire balls | | | | | | Bioassays performed using cones | | | | | |
|-----------|--------------------------------------|------------------|----------|-------|-------------|-----------|---------------------------------|----------|-------|-------------|-------------|-------------|
| | No. Exposed | No. knocked down | No. dead | % KD | % mortality | % Exposed | No. knocked down | No. dead | % KD | % mortality | No. exposed | % mortality |
| Control | | | | | | | | | | | | |
| 1 | 110 | 6 | 7 | 5.5 | 6.4 | 100 | 2 | 3 | 2.0 | 3.0 | | |
| 2 | 165 | 0 | 1 | 0.0 | 0.6 | 150 | 1 | 1 | 0.7 | 0.7 | | |
| 4 | 110 | 3 | 5 | 2.7 | 4.5 | 100 | 3 | 10 | 3.0 | 10.0 | | |
| 5 | 55 | 0 | 2 | 0.0 | 3.6 | 50 | 0 | 1 | 0.0 | 2.0 | | |
| 6 | 55 | 0 | 1 | 0.0 | 1.8 | 50 | 0 | 2 | 0.0 | 4.0 | | |
| ® | | | | | | | | | | | | |
| 1 | 110 | 108 | 110 | 98.2 | 100.0 | 100 | 100 | 97 | 100.0 | 97.0 | | |
| 2 | 165 | 165 | 146 | 100.0 | 88.5 | 150 | 139 | 75 | 92.7 | 50.0 | | |
| 4 | 110 | 110 | 106 | 100.0 | 96.4 | 100 | 95 | 77 | 95.0 | 77.0 | | |
| 5 | 55 | 55 | 48 | 100.0 | 87.3 | 50 | 46 | 18 | 92.0 | 36.0 | | |
| 6 | 55 | 55 | 34 | 100.0 | 61.8 | 50 | 50 | 23 | 100.0 | 46.0 | | |
| Olyset® | | | | | | | | | | | | |
| 1 | 110 | 108 | 103 | 98.2 | 93.6 | 100 | 98 | 86 | 98.0 | 86.0 | | |
| 2 | 165 | 165 | 114 | 100.0 | 69.1 | 150 | 104 | 39 | 69.3 | 26.0 | | |
| 4 | 110 | 104 | 93 | 94.5 | 84.5 | 100 | 87 | 74 | 87.0 | 74.0 | | |
| 5 | 55 | 55 | 38 | 100.0 | 69.1 | 50 | 45 | 12 | 90.0 | 24.0 | | |
| 6 | 55 | 40 | 19 | 72.7 | 34.5 | 50 | 31 | 9 | 62.0 | 18.0 | | |
| PermaNet® | | | | | | | | | | | | |
| 1 | 110 | 108 | 108 | 98.2 | 98.2 | 100 | 97 | 93 | 97.0 | 93.0 | | |
| 2 | 165 | 165 | 156 | 100.0 | 94.5 | 150 | 150 | 124 | 100.0 | 82.7 | | |
| 4 | 110 | 107 | 110 | 97.3 | 100.0 | 100 | 91 | 79 | 91.0 | 79.0 | | |
| 5 | 55 | 55 | 49 | 100.0 | 89.1 | 50 | 46 | 25 | 92.0 | 50.0 | | |
| 6 | 55 | 55 | 51 | 100.0 | 92.7 | 50 | 50 | 42 | 100.0 | 84.0 | | |

*The control consists of non insecticidal nets. Knock-down was assessed 60 minutes after exposure while mortality was assessed after 24 hours of exposure. No bioassays were conducted in the third month due to lack of mosquitoes.

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PART THREE

Preview of Part Three

This part of the thesis consists of three chapters:

Chapter VI: Development of a mathematical model applicable for evaluating community level impacts of integrated malaria vector control. This chapter describes the first step in the development of a deterministic model which was later used to simulate community level effects of combining long lasting insecticide treated nets (LLINs) with indoor residual spraying (IRS), as described in Chapter VIII. At this initial stage, this model version was tested by simulating combinations of LLINs with odour baited mosquito traps.

Chapter VII: Improvement and further testing of the mathematical model developed in chapter VI. This chapter describes the second stage of the mathematical modelling work. It includes a series of incremental improvements that were made to the model described in Chapter VI, so that it would be more representative of mosquito life cycle processes, and how these processes can be affected by different LLIN and IRS applications, with different modes of action. After these improvements, the model was tested by simulating effects of insecticidal applications which primarily repel malaria transmitting mosquitoes versus those, which primarily kill the mosquitoes.

Chapter VIII: Simulated community level effects of combining LLINs with IRS for malaria vector control in Africa: This chapter describes the final stages of the mathematical modelling work. In addition to some additional improvements, this chapter effectively describes the actual application of the fully developed model as described in Chapters VI and VII, to assess community level effects of combining LLINs with IRS. Data used for this specific simulation originated from the field study described in Chapter IV.

Important Note: Regarding the LLINs referred to in Chapters VI to VIII as **Icon Life®** nets, the supplier (Syngenta ltd) informed us at the end of our studies that this net type is the same as the one branded as **NetProtect®**, which has actually been given an interim approval by WHO (<http://www.who.int/whopes/quality/en>). However, in this thesis, the brand name **Icon Life®** has been retained, given that this was the label on the actual nets that we evaluated in the studies described here.

Chapter VI

Potential benefits, limitations and target product-profiles of odour-baited mosquito traps for malaria control in Africa^{*}

Abstract

Background: Traps baited with synthetic human odors have been proposed as suitable technologies for controlling malaria and other mosquito-borne diseases. We investigated the potential benefits of such traps for preventing malaria transmission in Africa and the essential characteristics that they should possess so as to be effective.

Methods and principle findings: An existing mathematical model was reformulated to distinguish availability of hosts for attack by mosquitoes from availability of blood *per se*. This adaptation allowed the effects of pseudo-hosts such as odour-baited mosquito traps, which do not yield blood but which can nonetheless be attacked by the mosquitoes, to be simulated considering communities consisting of users and non-users of insecticide-treated nets (ITNs), currently the primary malaria prevention method. We determined that malaria transmission declines as trap coverage (proportion of total availability of all hosts and pseudo hosts that traps constitute) increases. If the traps are more attractive than humans and are located in areas where mosquitoes are most abundant, 20-130 traps per 1000 people would be sufficient to match the impact of 50% community-wide ITN coverage. If such traps are used to complement ITNs, malaria transmission can be reduced by 99% or more in most scenarios representative of Africa. However, to match cost-effectiveness of ITNs, the traps delivery, operation and maintenance would have to cost a maximum of US\$4.25 to 27.61 per unit per year.

^{*} Adapted from: Okumu FO, Moore SJ, Govella NJ, Chitnis N, Killeen GF: *Potential benefits, limitations and target product-profiles of odor-baited mosquito traps as a means of malaria control*. PLoS ONE 2010, 5:e11573

Conclusions and significance: Odor-baited mosquito traps might potentially be effective and affordable tools for malaria control in Africa, particularly if they are used to complement, rather than replace, existing methods. We recommend that developers should focus on super-attractive baits and cheaper traps to enhance cost-effectiveness, and that the most appropriate way to deploy such technologies is through vertical delivery mechanisms.

Background

The interactions between mosquitoes and humans are central to the transmission of human malaria and other mosquito borne pathogens. Blood-seeking mosquito vectors identify humans from more than 30 meters away by detecting and following the chemical cues that the humans emit [1,2]. In recent years, studies of the olfactory mechanisms of the *Anopheles* mosquitoes, which transmit malaria in Africa, have yielded considerable insights into the molecular and physiological processes involved [3]. In some studies, the aim has been to discern how these processes influence malaria transmission [4,5], while in others it has been to find synthetic compounds that attract or repel mosquitoes [6-9]. From a public health point of view, the primary motive for investigating these issues lies in the potential to create new mosquito surveillance and abatement technologies.

While their applications in public health are still limited, odor-baited technologies are widely exploited in the agricultural sector where pest control is generally more advanced than is the case for vectors of human diseases [10]. Notable examples of success include the push-pull strategies practiced in crop pest management [11-13] and the control of tsetse flies, which transmit human and animal trypanosomiasis [14-16]. In both cases, the behavior of the pest is manipulated such that, instead of finding their intended hosts, they are lured into traps or onto insecticide-treated targets. Several types of odor-baited mosquito traps have been developed but they are used primarily for sampling, rather than controlling vector populations. Common examples include traps baited with whole humans [17-21], and those baited with carbon dioxide or other synthetic host cues [22-27]. Perhaps the most convincing examples of what may be possible by introducing lethal traps or targets is provided by the most successful existing methods of malaria control today:

Insecticide-treated nets (ITNs) [28,29] and the application of indoor-residual sprays (IRS) to houses [30,31]. Both methods essentially turn existing blood resources (people) and associated resting site resources (human dwellings) into lethal mosquito traps.

One important factor to consider before introducing new vector control methods, such as odor-baited mosquito traps, in Africa is the ongoing scale up of long lasting insecticidal nets (LLINs) across the continent [29]. These nets have lowered malaria burden in many endemic countries [28,32,33] and are currently prioritized as the frontline malaria prevention method across most of Africa [34-36]. Moreover, past and recent trends indicate that many countries are steadily increasing coverage with ITNs [29,37]. With these developments, it is necessary that any new tools are not evaluated in isolation, but rather on the basis of how much additional benefit they confer upon these communities where nets are already being used. The successful rollout of ITNs also poses new challenges by selectively suppressing transmission by indoor biting mosquitoes that prefer human blood [38]. New complementary vector control strategies that target the more zoophagic, exophagic vector species are required to tackle the residual transmission mediated by such modified vector populations.

While some relatively expensive designs have been proposed as being suitable for trapping mosquitoes in numbers sufficient to achieve population control [25,27,39,40], no rigorous large scale and independent evaluations of these technologies have been reported. More importantly, even though there is a constantly growing interest in odor-baited technologies, essential characteristics which they should possess so as to effectively control or disrupt malaria transmission have not been determined. Also unknown are the optimal approaches that could be used to

deliver them as public health commodities. Nevertheless, recent field trials of novel synthetic odor blends have shown that they can exceed the attractiveness of humans by up to four fold [41] and affordable, practical outdoor trap designs are becoming available [40,42], so the possibility of controlling malaria vector populations and malaria transmission is becoming increasingly realistic.

Here, the potential for using odor-baited mosquito traps to control malaria in a number of common epidemiological scenarios in Africa is mathematically investigated. Firstly, we examined whether traps, when used alone or as a complementary intervention alongside insecticidal nets, can fully reduce malaria transmission in highly endemic areas. Secondly, the target product-profiles that developers of this technology should consider so as to ensure effectiveness under real-life operational conditions were elucidated. These were accomplished by modifying an existing mathematical model of malaria transmission [43], which has previously been useful for informing global ITN coverage policy [36], but for which substantive revision was prompted by this particular example of odor-baited mosquito traps. The traps were treated as pseudo-hosts, which unlike humans or cattle, cannot provide blood to host-seeking mosquitoes, but which mosquitoes can attack nonetheless. This conceptual reformulation enabled explanation of the potential value and target product profiles of mosquito traps as a means to complement ITNs.

Methods

Description of the model

This is an adaptation of a deterministic model representing the most important host-seeking, survival and malaria transmission processes that individual mosquitoes

undertake before they can transmit malaria [43]. All parameter symbols and their meanings are outlined in Tables 1 and 2. Versions of the original model have been used to explore effects of bednets, cattle, repellents and insecticides on malaria transmission [44], to outline global coverage targets [36] and likely efficacy of ITNs [45], and also to examine interactions within push-pull strategies such as combining net-use with zooprophylaxis using cattle [46].

Blood feeding is the most important epidemiological event in the interactions between humans and malaria vector mosquitoes [47,48]. In this model, the blood acquisition process is considered as having three phases: 1) the mosquito being in a host-seeking state, 2) the mosquito attacking the host (or diverting away) and 3) the mosquito feeding upon the host (Figure 1). As in previous works by other authors, this feeding process is considered to be cyclical rather than continuous, so as to more accurately represent natural events [49,50-52]. The model examines diversion and mortality processes that occur during the three phases and how changes induced by interventions upon these processes can contribute to individual and community-level protection against malaria.

Effects of odor-baited traps were simulated in conceptual environments of two alternative dominant vector species (*Anopheles gambiae sensu stricto* Giles or *An. arabiensis* Patton) [53] in the presence of cattle, the main alternative blood source for these vectors [54], and presence or absence of ITNs. In each test scenario, the technology was evaluated in terms of combined, individual and community-level protection against malaria transmission when traps are implemented alone or in combination with ITNs.

Similar to most malaria transmission models, an enclosed ecosystem of parasites, vectors and hosts, is assumed [55,56]. In order to further reduce

computational complexity, the human hosts are considered to be homogenously mixed, meaning that vulnerability of individuals to malaria infection [5,57] or attractiveness of individuals to mosquitoes [2,58,59] can be reasonably estimated using population mean values for these parameters. These assumptions allowed for exploration of what might be possible if the traps are concentrated in geographical areas where mosquito densities are most abundant. Such locations are known to exist in real field settings [60-62] and can be targeted to achieve greatly enhanced control of pathogen transmission [63].

In the original model, the term ‘hosts’ referred to any vertebrate blood-sources upon which vectors can feed. This definition is hereby expanded to include all entities that a vector can attack with the intention of taking a blood meal, regardless of whether that entity actually has blood or not. This redefinition allows for inclusion of odor-baited traps as additional hosts (more precisely, pseudo-hosts) even though mosquitoes cannot possibly obtain blood from them. Another modification was a more explicit sub-division of the host-seeking process. Unlike the original model, the host-seeking process is considered here as consisting of two successive stages leading to the mosquito attacking the host namely: 1) non-host oriented *kinesis*, referring to arbitrary movements of the mosquito before it detects host cues, a process which ends with a host encounter event, and 2) host-oriented *taxis*, referring to directional movements of the mosquito once it encounters and detects the host cues in the environment and starts moving towards the source of those cues, a process which if initiated, either ends with a host attack event, or is aborted resulting in diversion back to *kinesis* (Figure 1).

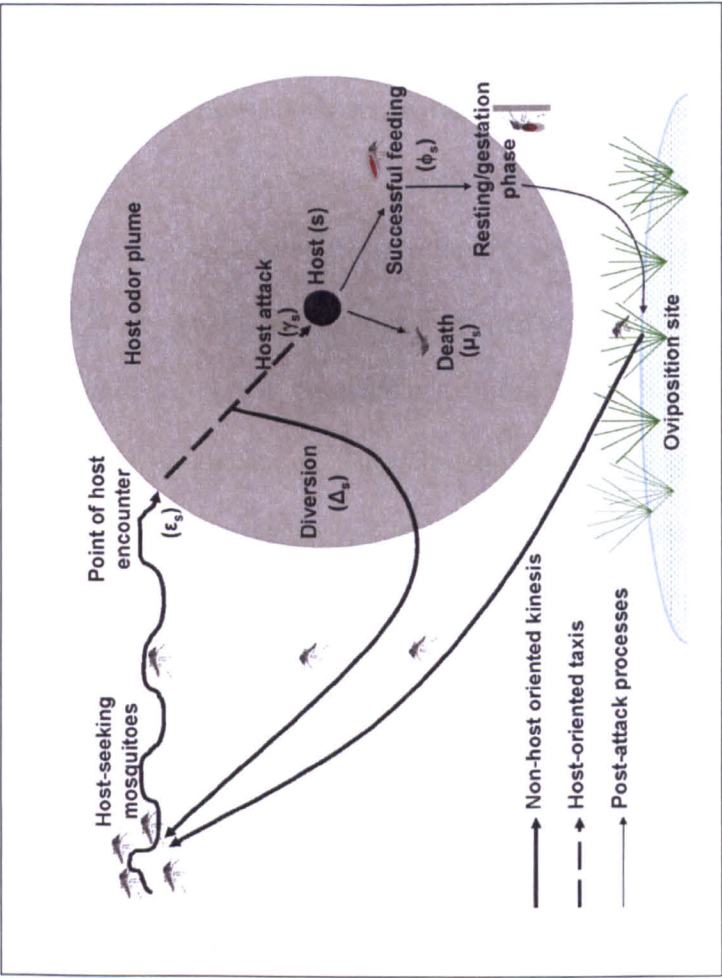


Figure 1: A simplified conceptual structure of the adapted model. This figure shows behavioral and mortality processes that occur in a mosquito feeding cycle. The host-seeking process includes *non-host oriented kinesis* and *host-oriented taxis*. The gray circular area represents extent of detectable odor plume around a host of species or type (s). In blood acquisition processes, mosquitoes are said to encounter hosts when they first detect odor cues associated with that host (ϵ_s). Then they can either attack the encountered host (γ_s) or be diverted back to non-host-oriented kinesis (Δ_s). Mosquitoes which go on to attack the host can either successfully feed (ϕ_s) or die (μ_s). Mosquitoes which successfully feed will go on to rest, digest the blood meals and then oviposit their eggs before eventually returning to host seeking state. This diagram is not drawn to scale and the host odor plume may not always be circular.

The duration of non-host-oriented kinesis, which is equivalent to the reciprocal of the rate at which an individual host is encountered by an individual vector, depends on: 1) physical distance between hosts and mosquitoes and 2) the distance over which attractive host odor plumes can extend. This means mosquitoes are more likely to encounter hosts which are near to the point at which they began host-seeking than those hosts which are far away. In nature, such spatial relations, including modifiers such as topography and wind direction are known to be important determinants of rates at which individual hosts are encountered [60-65].

This definition of the kinesis process also means that mosquitoes will more readily encounter hosts whose odor plumes extend over a wide radius than hosts which have short-radius plumes. For the purposes of this model, wider odor plumes are regarded as being equivalent to more mosquitoes potentially falling within the range of host encounter. Therefore hosts generating such kairomonal plumes are considerably more readily available than hosts generating less dispersed, short radius plumes. Interestingly, recent field trials of odor-baited traps demonstrate that the host-specific cues which malaria vector mosquitoes use to identify their preferred human hosts act mainly as long range attractants, presumably triggering the encounter process itself and allowing mosquitoes to make the choice between attack and diversion as early and as efficiently as possible [41].

Host-oriented taxis begins immediately after host encounter once the mosquito has chosen to proceed with host attack. There is a possibility that a mosquito encountering a non-preferred host type will ignore the opportunity to approach the host or may discontinue taxis, thus diverting back to non-host-oriented kinesis to seek other hosts. Once the mosquito commits to attack a host, it is assumed to complete a full taxis phase which ends with the host attack event.

The original definition of host availability [43] was also altered to specifically and separately describe the availability of hosts for attack rather than availability of host blood *per se*. The availability (a) of any host of any species or type (s) for mosquitoes to attack is the product of the rate at which individual vectors encounter that host (ε_s) and the probability that, after this encounter, they will attack the host (γ_s):

$$(1) \quad a_s = \varepsilon_s \gamma_s$$

Previously, host availability had been described as the product of host encounter rate and feeding probability [43,44,46,54]. Replacing the term, *feeding* with the term, *attack*, allows us to model the behavior of mosquitoes which attack the odor-baited traps and for which the feeding probabilities are therefore nil. A closer examination of what was previously defined as host availability [43] reveals that actually, it represents the availability of host blood at a particular source rather than the availability of the hosts themselves. That is to say, the availability of host blood (z) from a host of any species or type (s) is the product of the rate at which individual vectors encounter this host (ε_s) and the probability that, after this encounter, they will successfully feed upon that particular host (ϕ_s):

$$(2) \quad z_s = \varepsilon_s \phi_s$$

Similar to the original model, we label certain parameters with subscript s to represent different host species or host types including humans, cattle or odor-baited traps. Also, where necessary, the subscript s is specified as one of three different subscripts, t , c , h to represent traps, cattle and humans respectively. Moreover, humans not using nets (unprotected humans) and humans using nets (protected humans) are in some cases specifically represented by subscripts h,u and h,p respectively. Another subscript, j , which was used in previous versions of the original

model [43,44] to represent individuals within different host types or species, has been omitted in this reformulation, as no specific individual hosts are considered and instead, all parameters in this paper represent mean values for respective host populations.

When the mosquito encounters the host, it can either attack the host (successfully completing the host-seeking process, but not necessarily the blood acquisition process) or it can be diverted from the host (aborting the host-seeking process). The attack (γ_s) and diversion (Δ_s) probabilities therefore sum to unity.

$$(3) \quad \gamma_s + \Delta_s = 1$$

After host encounter, all diverted mosquitoes are assumed to re-enter non-host-oriented kinesis afresh. The diversion may include behavioral responses of mosquitoes to non-preferred or protected hosts which prompt them to abort taxis. For preferred hosts, diversion may be induced by physical barriers like house screens and untreated nets or chemicals used to treat nets or houses, and which repel or irritate mosquitoes [66,67].

However, not all vectors that attack the host will successfully feed. To account for mosquitoes that die during this attack process, a term for the mean attack-related mortality (μ_s) is introduced. It is assumed that only two possibilities exist at this stage: either the vector feeds successfully and consequently survives or it dies in the attempt before obtaining a blood meal. All mortality risks associated with host attack are expressed as a single mean probability and assumed to occur prior to feeding. The probability of successful feeding per host encounter (ϕ_s) is therefore calculated as follows:

$$(4) \quad \phi_s = \gamma_s(1 - \mu_s) = (1 - \Delta_s)(1 - \mu_s)$$

Assuming similar levels of baseline host defensiveness, the probabilities of diversion (Δ) and attack related mortality (μ) are considered to be same for cattle (c) and humans who are not using ITNs, i.e. unprotected humans (h,u). Equation 4 can therefore be specified as follows:

$$(5) \quad \phi_c = \phi_{h,u} = \gamma_{h,u}(1 - \mu_{h,u}) = (1 - \Delta_{h,u})(1 - \mu_{h,u})$$

Personal and house-hold protection measures such as bednets, repellents or domestic insecticides function by diverting host-seeking vectors or killing the vectors. The terms, Δ and μ are therefore modified for ITN users i.e. protected humans (h,p), to become $\Delta_{h,p}$ and $\mu_{h,p}$ respectively. Consistent with Killeen & Smith (2007) [44], the new terms are obtained by adding the ITN-induced changes to the baseline diversion and baseline mortality values:

$$(6) \quad \Delta_{h,p} = \Delta_{h,u} + \pi_i \theta_\Delta (1 - \Delta_{h,u})$$

$$(7) \quad \mu_{h,p} = \mu_{h,u} + \pi_i \theta_\mu (1 - \mu_{h,u})$$

Where, θ_Δ and θ_μ represent the additional effects of ITNs on the diversion and mortality probabilities respectively. These coefficients were previously annotated as Δ_p and μ_p in the original model [43,44] but have now been changed to distinguish them more clearly from the $\Delta_{h,p}$ and $\Delta_{h,u}$, which refer to diversions from protected and unprotected humans respectively.

The term π_i in the two equations refers to the proportion of normal exposure to mosquito bites upon humans lacking ITNs that occurs during the times when nets would normally be in use [45,68]. It is used here to modify the terms θ_Δ and θ_μ , in order to obtain the true effects of ITNs upon a typical user. Without the term, π_i , the equations would represent merely an ideal situation where ITNs are consistently and correctly used over the full course of the time when malaria vectors bite. However, such an ideal scenario seldom happens and possessing a net does not always translate

to consistent and perfect use of it. Moreover, even the most nocturnal vectors can feed to some extent in the early evening hours before people go under their nets or in early mornings when many people are awake and are no longer protected [45,67,68].

Thus in practice, not all human exposure to mosquito bites occurs during the times when nets are actually in use [45,67-69]. Note that this approach deals more simply and parsimoniously with such behavioral avoidance of interventions, than previous approaches by incorporating these effects at the single point of the model where they actually act in biological reality, rendering the more elaborate and indirect formulations such as equation 8 in Killeen *et al.*, 2007 [43] and equation 1 in Govella *et al.*, 2010 [45], redundant.

Equations 6 and 7 are used to specify equation 4 in order to explicitly express the probability of successful feeding upon an ITN user ($\phi_{h,p}$):

$$(8) \quad \phi_{h,p} = \gamma_{h,p}(1 - \mu_{h,p}) = (1 - \Delta_{h,p})(1 - \mu_{h,p})$$

Table 1: Symbols and their meanings

| Symbol | Definition | References |
|---------------|--|-------------------|
| a | Availability of individual hosts: rate at which a single mosquito encounters and then attacks a given single host or pseudo-host. | This paper. |
| A | Total availability of hosts and pseudo hosts: rate at which a single mosquito encounters and attacks all hosts and pseudo hosts. | This paper. |
| β | The mean number of infectious bites per emerging mosquito during its lifetime. | [43,44,73]. |
| c | Cattle. | [43,44]. |
| C_A | Proportion of the total available host resources accounted for by the odor-baited traps, equivalent to trap coverage. | This paper. |
| C_h | Proportion of people using ITNs, equivalent to ITN coverage as surveyed by its most relevant indicator [117]. | [43,44]. |
| Δ | Probability that a mosquito which encounters a host will be diverted from that host. | [43,44]. |
| ε | Host-encounter rate: rate at which a single host-seeking mosquito encounters a given single hosts. | [43,44,54]. |
| E | Emergence rate of mosquito vectors per year. | [43,44,73]. |
| EIR | Entomological inoculation rate (mean number of infectious bites that an average individual human receives per year). | [43,44,54,73,77]. |
| ϕ | Probability that a mosquito which attacks a host will successfully feed upon that host. | [43,44,54]. |
| f | Feeding cycle length: measured as the number of days it takes a single mosquito to get from one blood feed to the next. | [43,73]. |
| g | Gestation interval: number of days a mosquito takes to digest a blood meal and return to searching for oviposition site. | [43,44]. |
| h | Humans. | [43,44]. |
| h,p | Protected humans using ITNs. | [43,44]. |
| h,u | Unprotected humans not using ITNs. | [43,44]. |
| κ | Human infectiousness to mosquitoes: probability of a vector becoming infected per human bite. | [43,49,73]. |
| λ | Relative availability of hosts other than humans: calculated as a ratio of availability of those hosts to availability of humans not using ITNs. | [41,43,54]. |
| L | Potential of any individual vector to transmit malaria from infectious humans over its lifetime. | [73]. |
| μ | Probability that a mosquito which attacks a host will die during the attack. | [43,44]. |
| η_o | Oviposition site-seeking interval: number of days that a mosquito takes to find an oviposition site once it starts searching for it. | [43,44]. |
| η_v | Host-seeking interval: number of days a mosquito takes to find and attack a host. | [43,44,54]. |
| N | Number of hosts. | [43,44]. |
| θ_d | Excess proportion of mosquitoes which are diverted while attempting to attack a human while that person is using an ITN. | This paper. |

Table 2: Symbols and their meanings-continued from table 1

| Symbol | Definition | References |
|---------------------|--|----------------|
| θ_μ | Excess proportion of mosquitoes which die while attempting to attack a human while that person is using an ITN. | This paper. |
| Ω | Intervention package scenarios consisting of a specific coverage with ITNs and a specific number of odor-baited mosquito traps per 1000 people. | This paper. |
| π_i | The proportion of normal exposure to mosquito bites upon humans lacking ITNs, which occurs indoors at times when nets would normally be in use. | [43,45,68]. |
| P | Probability that a resting mosquito survives any one day. | [43,44]. |
| P_f | Probability that a mosquito survives a single complete feeding cycle. | [43,44]. |
| P_{ov} | Probability that a mosquito survives any full day of the oviposition site-seeking interval or host-seeking interval. | [43,44]. |
| Q_h | Human blood index: the proportion of all blood meals from all hosts and pseudo hosts, which are obtained from humans. | [43,44,54,73]. |
| s | Host species or host type | [43,44]. |
| t | Odor-baited mosquito traps. | This paper. |
| γ | Probability that a mosquito attacks an encountered host. | |
| ψ | Relative exposure of different hosts other than unprotected humans to mosquito bites: calculated as a ratio of exposure of those hosts to exposure of humans not using nets. | This paper. |
| $\psi_{h,p,\Omega}$ | Combined personal and communal protection provided by the integrated intervention package Ω to people who use ITNs. | This paper. |
| $\psi_{h,Traps}$ | Additional protection offered by odor-baited traps to communities using ITNs. | This paper. |
| $\psi_{h,u,\Omega}$ | Communal protection provided by the integrated intervention package Ω to people who do not use ITNs. | This paper. |
| ψ_Ω | Mean relative exposure of an average member of a community where the intervention package Ω is implemented. | This paper. |
| z | Availability of blood from an individual host: rate at which a single mosquito encounters, attacks and successfully feeds upon a given single host | This paper. |
| Z | Total availability of blood from hosts and pseudo hosts: rate at which a single mosquito encounters, attacks and successfully feeds upon all hosts. | This paper. |

Modeling the effects of individual odor-baited traps

Odor-baited traps are assumed to affect the foraging behavior of host-seeking mosquitoes by triggering the transition from kinesis to taxis, in exactly the same way as vertebrate hosts. Their efficacy as tools to control malaria transmission is derived primarily from two complementary characteristics: 1) their high attractiveness to malaria mosquitoes compared to attractiveness of humans [41] and 2) their ability to trap and kill mosquitoes which attack them thus removing these mosquitoes from the biting population. Any given trap type can therefore be described in terms of its mean availability for attack by host-seeking mosquitoes (a_i), defined as the rate at which it is encountered (ε_i), and the probability that it is attacked by the mosquitoes (γ_i) following encounter. As successful blood feeding upon a trap is not a possible outcome, the mortality probability for mosquitoes that attack a trap (μ_i) and the corresponding probability of successful blood feeding (ϕ_i), are fixed at one and zero respectively ($\mu_i=1$, $\phi_i=0$).

These assumptions about individual-level processes enable adaptation of subsequent equations from the original formulation [43], so as to estimate population-level effects of odor-baited traps used alone or in combination with ITNs, and also to elucidate desirable characteristics of such devices.

Estimating population level effects of odor-baited traps when used alone or in combination with ITNs

The availabilities of cattle (a_c) and traps (a_i) for attack by host-seeking mosquitoes were calculated based on field estimates of their relative availabilities (λ_c for cattle

[54] and λ_t for odor-baited traps [41]) when compared to the availability of humans for similar attacks as described in equation 1:

$$(9) \quad \lambda_c = \frac{a_c}{a_h}$$

$$(10) \quad \lambda_t = \frac{a_t}{a_h}$$

For any given number of odor-baited traps (N_t), cattle (N_c), people not using ITNs ($N_{h,u}$) and people using ITNs ($N_{h,p}$), the total host availability (A) was calculated as the sum of the products of mean availabilities of each host species or type (a_s) and the number of hosts of that particular species or type (N_s). However, unlike in the original formulation [43], the term host availability hereby includes events only up to host attack, thus excluding all probabilities of blood feeding or death after the attack. The mean host-seeking interval (η_v) was then calculated as the reciprocal of total host availability (A) and consistent with previous formulations [54]:

$$(11) \quad \eta_v = \frac{1}{A} = \frac{1}{A_h + A_c + A_t} = \frac{1}{a_{h,u}N_{h,u} + a_{h,p}N_{h,p} + a_cN_c + a_tN_t}$$

The relative exposure of any host to mosquito bites (which is calculated as a function of successful feeding and therefore the availability of blood rather than hosts *per se*) is therefore no longer equivalent to its relative availability when calculated as a function of host attack probability. This means that any two hosts can be equally available for attack but may be differentially exposed if interventions which cause different levels of reduction of successful feeding despite equal levels of diversion are specified. The relative exposure (ψ) of different hosts must therefore be calculated separately from relative availability of attackable hosts and must be based on the availability of the blood resource that each host type or species (s) represents to

mosquitoes (z_s). For example, relative exposure of humans protected with ITNs, when compared to that of humans not protected with ITNs is calculated as follows:

$$(12) \quad \psi_{h,p} = \frac{z_{h,p}}{z_{h,u}} = \frac{\phi_{h,p}}{\phi_{h,u}}$$

where $z_{h,p}$ refers to the mean availability of blood from a protected human.

For a vector to complete one feeding cycle, it must survive all the host-seeking phases shown in Figure 1 including gestation to convert blood to eggs and then an equivalent set of resource acquisition processes required to enable oviposition. While gestation is primarily spent resting in relatively safe places, which are often inside houses, foraging for resources is an intrinsically dangerous process for mosquitoes. Even without any human intervention, survival is reduced by numerous biotic and abiotic factors in the environment such as predators, host defensive behavior and dehydrating conditions of heat and low humidity [70,71].

As in our original model [43] and in some previous models by other authors [50,72], it was assumed that survival during host-seeking and oviposition site-seeking phases is lower than survival while the mosquito is resting inside houses. Survival across all phases of the gonotrophic cycle was estimated as the distinct daily survival probability during each phase to the power of the respective time intervals, namely the host-seeking interval (η_v), gestation interval (g) and oviposition site-seeking interval (η_o). Though the current definition for host-seeking refers to processes up to and including attack, but not blood acquisition itself, the duration between the time when the mosquito attacks the host and the time when it bites and acquires blood from it, is considered here to be a negligible interval in the context of a gonotrophic cycle which lasts for two or more days.

The daily survival probability of a resting mosquito is defined as P and the survival probabilities during host-seeking and oviposition site-seeking are assumed to

be equal and are both defined using the term (P_{ov}). The survival rate per feeding cycle (P_f) was therefore estimated as the combined probability that a vector survives gestation (P^g), oviposition site-seeking ($P_{ov}^{\eta o}$), host-seeking ($P_{ov}^{\eta v}$) and the eventual attack of a host (P_γ):

$$(13) \quad P_f = P^g P_{ov}^{\eta o} P_{ov}^{\eta v} P_\gamma = P^g P_{ov}^{\eta v + \eta o} P_\gamma$$

To calculate the probability of mosquitoes surviving their eventual attack upon any host (P_γ), we assumed that the proportion of all attacks that end in death is the mean of the mortality probabilities for attacking the various hosts (non-ITN users, ITN users, cattle or odor-baited traps), weighted according to the proportion of total availability that each host class represents [45]:

$$(14) \quad P_\gamma = 1 - \frac{\mu_{h,p} a_{h,p} N_{h,p} + \mu_{h,u} (a_c N_c + a_{h,u} N_{h,u}) + a_u N_t}{a_{h,u} N_{h,u} + a_{h,p} N_{h,p} + a_c N_c + a_u N_t}$$

This term differs slightly from equation 13 of the original formulation [43], in that it now reflects ITN effects that have been modified by the proportion of normal unprotected human exposure that occurs during times when this intervention would typically be in use (π_i) [45,68], but does so more directly than the more complex formula of Govella *et al.*, 2010 [45] because this effect has already been captured by equations 6 and 7. The term for mortality upon attacking an odor-baited trap (μ_t) could be included explicitly in the numerator so that the equation is clearer, but because it has already been defined as being equal to one, the trap terms in both the numerator and denominator are expressed simply as $a_t N_t$. Here again, this revised formulation is more specific and predicts survival of attack based only on rates of attack rather than the probabilities of successful feeding.

The human blood index (proportion of all blood-meals that originate from humans; Q_h), was calculated based on the proportion of the total availability of blood

from all host types (Z), which humans represent (Z_h). Note that for any host species or type, $Z_s = z_s N_s$. Specifically, Q_h was therefore calculated as the proportion of surviving mosquitoes obtaining a blood meal that do so from humans, based upon the overall total rates of encounter of each host type and the probabilities of successfully obtaining a blood meal from each:

$$(15) \quad Q_h = \frac{Z_{h,u} + Z_{h,p}}{Z_{h,u} + Z_{h,p} + Z_c + Z_t}$$

$$(16) \quad = \frac{z_{h,u} N_{h,u} + z_{h,p} N_{h,p}}{z_{h,u} N_{h,u} + z_{h,p} N_{h,p} + z_c N_c + z_t N_t}$$

$$(17) \quad = \frac{\epsilon_h (N_{h,u} \phi_{h,u} + N_{h,p} \phi_{h,p})}{\epsilon_h (N_{h,u} \phi_{h,u} + N_{h,p} \phi_{h,p}) + \epsilon_c N_c \phi_c}$$

It should be noted that equation 17 also does not contain terms for odor-baited traps (N_b , ϵ_t and ϕ_t) in the denominator. This is because it is impossible for mosquitoes to obtain blood meals from the traps so even if the term ϕ_t were included, it would be valued zero thus rendering the equation mathematically equivalent to the above.

Estimating protection against exposure to malaria

As described in the very first formulation of the population-level component of this hierarchical model [73] and its subsequent improvements [43,44], the survival rate per feeding cycle (P_f) and the proportion of blood meals taken from humans (Q_h) were used to calculate the potential of any individual vector to transmit malaria from infectious humans over its lifetime (L). The term L together with human infectiousness to mosquitoes (κ) were then used to calculate the mean number of infectious bites per emerging mosquito during its lifetime (β). To obtain the sum of all infectious bites that occur in the whole human population, the mean number of

infectious bites per emerging mosquito (β) was multiplied by the emergence rate of mosquito vectors (E). If this product (βE) is divided by the human population size (N_h), we obtain the mean number of infectious bites that an average individual human receives, also referred to as the mean entomological inoculation rate (EIR) experienced by individuals in the community [73,74]:

$$(18) \quad EIR_h = \frac{\beta E}{N_h}$$

In a human population composed of two distinct subgroups (ITN users and non-users), it is important to calculate separately the EIR experienced by each subgroup so that we can compare them. For either subgroup, this is a product of the total number of infectious bites upon humans that occur in the population as a whole (βE) and the fraction of biting exposure experienced by that particular subgroup of the population. Here also, the original forms of these equations [43] are replaced with explicit forms to express the availability of blood rather than the availability of attackable hosts, and consequently capture exposure to bites rather than exposure to attacks:

$$(19) \quad EIR_{h,u} = \frac{\beta E \phi_{h,u}}{N_{h,u} \phi_{h,u} + N_{h,p} \phi_{h,p}}$$

$$(20) \quad EIR_{h,p} = \frac{\beta E \phi_{h,p}}{N_{h,u} \phi_{h,u} + N_{h,p} \phi_{h,p}}$$

For purposes of estimating the likely impacts of interventions, it is imperative to know how much the exposure to bites from malaria-infected mosquitoes can change when an individual becomes protected by a preventative measure such as an ITN. Dividing equation 20 by equation 19 and substituting with equation 12 provides a solution which is consistent with the commonly accepted definition of personal protection against exposure to infectious bites [68,75]:

$$(21) \quad EIR_{h,p} = \psi_{h,p} EIR_{h,u}$$

For integrated programs, involving the use of ITNs and odor-baited traps, there are several possible intervention package scenarios (Ω). Each package is explicitly defined by the ITN coverage (C_h), ITN properties (θ_Δ and θ_μ), number of odor-baited traps (N_i) and the mean availability (a_i) of those traps. For ease of comparison and interpretation, the impact of any intervention package, Ω , is expressed in terms of relative exposure to transmission intensity ($\psi_\Omega = EIR_\Omega / EIR_0$), where EIR_Ω is the mean exposure of humans in the presence of the intervention package and EIR_0 is the mean exposure of members of the same community when no intervention is present. We use the notation $EIR_0 = EIR_{h,u,0}$ to denote the EIR of all humans when no intervention is present, $EIR_{h,u,\Omega}$ to denote the EIR of humans without ITNs in a population with the intervention Ω and $EIR_{h,p,\Omega}$ to denote the EIR of humans with ITNs in a population with the intervention Ω . The mean EIR in the presence of the intervention package is therefore:

$$(22) \quad EIR_\Omega = C_h EIR_{h,p,\Omega} + (1 - C_h) EIR_{h,u,\Omega}$$

where C_h is the proportional coverage of the human population with ITNs.

The total benefits of any intervention package, Ω can then be apportioned to personal or communal protection benefits and expressed in terms of EIR relative to the baseline scenario with no interventions as follows:

$$(23) \quad \psi_{h,u,\Omega} = \frac{EIR_{h,u,\Omega}}{EIR_{h,u,0}} \quad \text{for communal protection provided by the integrated}$$

intervention package to people who do not use ITNs, and

$$(24) \quad \psi_{h,p,\Omega} = \frac{EIR_{h,p,\Omega}}{EIR_{h,u,0}} \quad \text{for combined personal and communal protection}$$

provided by the integrated intervention package to people who use ITNs.

Whereas people who do not use ITNs will benefit from only the communal protection provided by the integrated intervention package, those who use ITNs will benefit from both the personal protection provided by their own ITNs and the communal protection provided by the integrated intervention package. The contributions of personal and community-level protection to the benefits of ITNs have been discussed in detail elsewhere [43] and are therefore not the focus of this paper. Here, we express the influence of ITNs simply as the mean relative exposure of an average member of the community. This is calculated as the mean of the relative *EIR* of protected and unprotected hosts, weighted according to the proportions of the human population that they represent:

$$(25) \quad \psi_{\Omega} = \frac{EIR_{\Omega}}{EIR_0} = \psi_{h,u,\Omega}(1 - C_h) + \psi_{h,p,\Omega}C_h$$

When odor-baited traps are added to the intervention package alongside ITNs, we expect that the exposure of both net users and non-users to infectious mosquitoes is correspondingly reduced. Because ITNs are already widely used in Africa [29], the traps should be considered only as complementary interventions rather than as replacement for the ITNs. Their effects on transmission should therefore be evaluated in terms of the further transmission reductions they offer, relative to that which is provided by ITNs alone. To determine how much benefit the odor-baited traps would actually contribute towards the overall reductions generated by the combined intervention, the residual exposure experienced when the combined package is implemented is expressed relative to the residual exposure experienced when only nets at any given coverage (C_h) are used:

$$(26) \quad \psi_{h,Traps} = \frac{EIR_{\Omega}}{EIR_{\Omega - Traps}} \text{ reflecting additional protection offered by odor-}$$

baited traps to communities using ITNs.

Because odor-baited traps are considered as a distinct host type (more specifically pseudo-hosts), we used this model to explore the hypothesis that their effects on malaria transmission will depend on how much they contribute to the total availability of all hosts for attack by malaria mosquitoes, which is equivalent to the proportion of the total available host resources covered or accounted for by the odor-baited traps (C_A):

$$(27) \quad C_A = \frac{A_t}{A} = \frac{A_t}{\sum A_s} = \frac{A_t}{A_h + A_c + A_t}$$

It is expected that as C_A increases, so will the impact of the traps on malaria transmission. With reference to these reformulated equations there are two possible ways to increase total trap availability (A_t) and therefore increase C_A . These include increasing the relative availability of individual traps (λ_t) or increasing the number of traps deployed (N_t). Similarly, with reference to the current definition of mosquito host-seeking processes, the relative availability of individual traps (λ_t) can be increased by ensuring high encounter rates and high attack probabilities relative to that of the preferred vertebrate hosts such as cattle and humans. Practical ways to effect such enhancements are outlined explicitly in the section entitled *parameters describing odor-baited traps*.

Baseline ecological parameterization of the model

In Table 3, the ecological parameters and associated values used as well as the source references are outlined. As in the original model [43], a village with 1000 persons and 1000 head of cattle is considered. Parameter value for infectiousness of humans to mosquitoes (κ) was also set the same as in the original model (0.030). It was assumed that infectiousness of humans to mosquitoes is constant across the population,

regardless of the impacts of vector control measures. Therefore any additional benefit that may be accrued by reducing this parameter once EIR drops below the threshold of 10 infectious bites per person per year [76] is ignored. To achieve baseline transmission intensities representative of places in Africa where malaria transmission is constantly intense [77,78], we increased the mosquito emergence rate from the original value of 9 million [43] to 20 million, which resulted in baseline EIR values greater than 200 in the test scenarios, thus a typically challenging holoendemic scenario was represented.

The daily survival probability of a resting mosquito was set to 0.9 while the daily survival probability of mosquitoes while foraging for blood or oviposition sites (P_{ov}) was set to 0.80, also consistent with published applications of the original model formulations [43,44]. The baseline host defences of people who do not use ITNs, and of cattle, were assumed to be the same. Therefore, the probabilities for *An. arabiensis* and *An. gambiae s.s.* being diverted (Δ) or killed (μ) during attack on either non-ITN users or cattle was set as 0.1. This means 90% of all mosquitoes of these species would attack the hosts upon encountering them and thereafter 90% of those that attack the hosts will successfully take blood meals from them.

The mean availability of non ITN-users had been estimated for *An. arabiensis* on the basis of field estimates in a southern Tanzanian village at a time when less than 1% of the population used nets [79]. The study considered dissection based observations of the dilation status of ovariole stalks in host-seeking female mosquitoes caught with human-baited light traps [79]. The number of successful feeds per day per host-seeking vector per human was therefore originally calculated as the inverse of the inferred host-seeking interval of 0.7 days divided by the human population size in the study area, which was 1212 at that time [80].

Reconsidering this estimate in the light of this revised definition of host availability for attack, this approach to parameterization now seems even more appropriate as the dissected unfed mosquitoes were sampled during the attack phase, before feeding and obviously before death. In fact, the availability value used in the original model should actually have been defined as successful attacks (rather than successful feeds) per day per host-seeking vector. For the purposes of this new model formulation, the parameter value therefore remains unchanged and was applied also to *An. gambiae*. The mean availabilities of humans to *An. arabiensis* and *An. gambiae* were then used to calculate the mean availability of cattle to attack by the same vector species. Based on equation 9, this was accomplished by calculating the product of these mean availabilities (a_h) and estimates of the relative availability of cattle (λ_c), which had earlier been derived from field studies of mosquito host preferences [46,54]. Finally, the total availability of aquatic habitats (A_a) was set to 3, also unchanged from the previous application [43].

Table 3: Values and references for ecological parameters in the simulations ^a

| Definition | Symbol | Value | References |
|--|----------------|----------------------|-------------|
| Total number of cattle | N_c | 1000 | [43]. |
| Total number of humans | N_h | 1000 | [43]. |
| Diversion probability from an unprotected vertebrate host (cattle or human) | $\Delta_{h,u}$ | 0.1 | [43]. |
| Mortality probability upon attacking an unprotected host | $\mu_{h,u}$ | 0.1 | [43]. |
| Mean availability of individual unprotected humans ^b | $a_{h,u}$ | 1.2×10^{-3} | [43,54,79]. |
| Mean availability of individual cattle ^c | a_c | | [43,46,54]. |
| <i>An. arabiensis</i> | | 1.9×10^{-3} | |
| <i>An. gambiae</i> s.s. | | 2.5×10^{-5} | [43,46,54]. |
| Total availability of aquatic habitats | A_a | 3 | [43]. |
| Duration of gestation | g | 2 | |
| Proportion of mosquitoes surviving per day while feeding while resting | P | 0.9 | [43]. |
| Proportion of mosquitoes surviving per day while foraging for hosts or oviposition sites | P_{ov} | 0.8 | [43]. |
| Duration of the parasite sporogonic development period | n | 11 | [43]. |
| Human infectiousness to mosquitoes | κ | 0.03 | [43]. |
| Total number of adult mosquitoes emerging per year | E | 2.0×10^7 | This paper. |

^a This table contains only those ecological parameters considered to be necessary for the primary understanding and parameterization of the model. A full listing of all ecological parameters is available in Tables 1 and 2 and in file S1, within the spreadsheet containing the model. All entries refer to mean parameter values in this deterministic model.

^b The value of the parameter is equivalent to attacks per day per host-seeking vector per unprotected human.

^c The value of the parameter is equivalent to attacks per day per host-seeking vector per individual head of cattle and was different for the two vector species *Anopheles arabiensis* and *Anopheles gambiae sensu stricto*. With the exception of this parameter, all the other values are assumed to be identical for both species.

Parameters describing Insecticide Treated Nets

The intervention parameters and associated values used, as well as the source references, are outlined in Table 4. We considered baseline scenarios to be communities lacking traps but where ITNs were either completely absent or being used by half of all age groups within the community. As in the original model, the effects of ITNs were quantified in terms of their ability to repel malaria vectors from humans and/or to kill the vectors whenever they attacked the net users. Though the World Health Organization, has to date approved seven different Long Lasting Insecticide Nets (LLINs), including interim approvals [81], we simulated scenarios with one long-lasting insecticidal net type, namely Olyset® nets, whose properties are representative of the most commonly used LLINs in Africa. These LLINs are knitted from polyethylene fibres that have been impregnated with a first-generation synthetic pyrethroid, namely permethrin [82-84]. Apart from being toxic to mosquitoes, permethrin is also an excito-repellent, meaning that the nets also divert considerable proportions of these mosquitoes even before they can attack net users [82-87]. The parameter values used in the simulation were chosen such that they approximate the properties of Olyset® nets under normal conditions of community use.

Repellency of nets, which is measured as a reduction in the number of mosquitoes that enter human-occupied huts [88] when the nets are used by the occupants, is reflected in the excess diversion of mosquitoes from an ITN user (θ_{Δ}). Correspondingly, the excess mortality upon attacking the ITN user (θ_{μ}) is estimated as the excess proportion of mosquitoes entering those experimental huts that die attempting to feed on the hut occupants, relative to control huts. The parameter values of the selected representative net type were set to reflect the following: 1) diversion of 50% of all mosquitoes that encounter the net users ($\theta_{\Delta} = 0.5$), and 2) excess mortality

of 70% of those mosquitoes attacking the net users ($\theta_{\mu}= 0.7$). These estimates were computed from reports of experimental hut studies previously conducted in the field [83-85,89,90]. As per equation 8, these diversion and mortality values mean that the nets would protect against 85% of all indoor malaria exposure (protection against bites = $100 \times (1-((1-0.5) \times (1-0.7)))$ %).

ITN coverage in Africa is gradually improving and an increasing number of countries are achieving net coverage of 50 % or more, especially for children under fives [29,37,91]. To achieve the full potential of nets, including valuable community-wide benefits, it is broadly agreed that reasonably high coverage of entire communities rather than just vulnerable groups is required [36,43,92,93]. Therefore, consistent with the best estimates of the minimum level community-wide coverage required [43,94], we simulated situations with 50% ITN use across all age groups to represent what is likely attainable in most African countries. In addition, we simulated situations with 80% ITN coverage to represent areas where ITN distribution and coverage in Africa have been highly successful and where existing net distribution and promotion mechanisms may guarantee such coverage levels [90].

Finally, the proportion of normal biting exposure of non-users that occurs indoors when nets would usually be in use (π_i) was set at 0.9 based on recent estimates for *An. gambiae sensu lato* from a malaria-endemic village in south eastern Tanzania [45,68].

Table 4: Values and references for intervention parameters in the simulations ^a

| Definition | Symbol | Value | References |
|--|------------------------|---------------------------|------------|
| Proportion of people using ITNs. | C_h | 0.001 ^b or 0.5 | This paper |
| Proportion of exposure that occurs indoors during the time when ITNs are actually in use. | π_i | 0.9 | [43,45,68] |
| Number of odor-baited mosquito traps. | N_t | varying | This paper |
| Additional diversions per ITN user encountered. | θ_Δ | 0.5 | This paper |
| Probability of mosquitoes being diverted from an odor-baited trap. | Δ_t | 0.1 | This paper |
| Probability of mosquitoes dying upon attacking an odor-baited trap. | μ_t | 1 | This paper |
| Additional mortality of mosquitoes per ITN user attacked. | θ_μ | 0.7 | This paper |
| Probability of mosquitoes successfully feeding upon an odor-baited trap. | ϕ_t | 0 | This paper |
| Relative availability of odor-baited mosquito trap to host seeking mosquitoes if the traps are placed homogenously among humans. | $\lambda_{t,unbiased}$ | 4 | [41] |
| Relative increase in availability of odor-baited mosquito traps achieved by spatially biasing position of the traps on the basis of 80-20 statistical distribution [63]. | $\lambda_{t,based}$ | 4 | This paper |

^a This table contains only those intervention parameters considered to be necessary for primary understanding and parameterization of the model. A full listing of all intervention parameters is available in tables 1 and 2 and in File S1, within the spreadsheet containing the model. All values represent mean parameter values in this deterministic model.

^b It is assumed that only one person among the 1000 people is using the ITNs

Parameters describing odor-baited trap technologies

A minimal diversion probability of 0.1 was assumed for mosquitoes encountering odor-baited traps, identical to baseline diversion probabilities from persons not using ITNs and also from cattle. Since there is no possibility of mosquitoes getting blood meals from the odor-baited traps, the probability of successful feeding upon the traps was set to be zero ($\phi = 0$). Correspondingly, because traps retain and kill the captured mosquitoes, we set the probability of attack-related mortality upon them to be one ($\mu_i = 1$). Considering the successive stages of host-seeking by a mosquito (Fig. 1), the relative availability of the traps (λ_i) could therefore be varied in different ways.

First, the encounter rate (ε_i) can be increased by making the traps easier for mosquitoes to find, either by placing them in locations close to breeding sites or by improving the attractants (baits) so that the range from which the traps are detected by host-seeking mosquitoes is extended. Moreover, changing the relative attractiveness of the traps to mosquitoes when compared to the attractiveness of actual human hosts, which is equivalent to changing attack probability (γ) could also lead to increased or reduced trap catches. However, given the very high attack probabilities assumed in this model, there is little scope for meaningfully increasing this parameter value. It is therefore likely that increasing encounter rates (ε_i) or the number of traps (N_i) are the primary means available to maximize total trap availability (A_i). We therefore hypothesize that these factors represent the key parameters that should be considered when outlining target product profiles for developers of odor-baited traps.

Few studies exist in which odor baits have been compared with humans under realistic field conditions. However in recent field evaluations in rural Tanzania, a mixture of synthetic attractants that mimic human odors, proved to be more attractive than humans to several genera of mosquitoes including malaria vectors [41]. These

experimental prototypes attracted approximately four times as many *Anopheles gambiae* as an average human whenever the traps and the human were in separate huts 15 to 100 meters apart, but the humans remained more attractive whenever the two were side by side inside the same hut, resulting in increased exposure of the humans to mosquito bites [41]. This indicates that the synthetic odor blend most probably acts as a long-range cue, attracting more mosquitoes to the point source, at which the mosquitoes then choose the co-located human host based on stronger short-range, non-host-specific stimuli such as heat and water vapor.

These field estimates were therefore used to compute the mean availability of individual traps (a_t) using equation 10 by simply multiplying mean availability of individual humans (a_h) by a factor of four ($\lambda_t = 4$). All the relevant intervention parameters and associated values are also outlined in Table 4.

Targeted positioning and delivery systems for odor-baited traps

By comparing the numbers of mosquitoes caught in huts where traps had been placed versus catches in huts where human volunteers slept [41], we estimated the relative availability of the odor-baited traps if such traps are evenly or randomly placed in a set of locations that are geographically distributed in the same way as the human population ($\lambda_t = 4$). In such a case of unbiased trap placement among human residences, encounter rates of the traps (ε_t) is simply a function of mean human availability (a_h) and the experimentally measured relative availability of traps (λ_t), which is primarily influenced only by the attractive range of those devices.

For ethical and safety reasons, however, odor-baited traps similar to the ones we have field-tested [40,41], should never be deployed in such a manner that they are evenly distributed among humans because they emit long-range attractants which can

increase exposure of nearby residents for the reasons described above (Sumaye *et al.*, Unpublished). In practice it is impossible to guarantee the minimum distances required to exclude this possibility in even the most modestly clustered human settlements. It is therefore essential that the odor-baited traps are placed far from human residences and aggregations thereof. Fortunately this also offers an excellent means to enhance intervention efficacy and minimize costs.

The targeted placement away from houses is desirable not only to maximize safety but also to take full advantage of mosquito distribution patterns, which naturally present significant opportunities to dramatically enhance effectiveness of mosquito trapping programs. Heterogeneities in the transmission of vector borne infectious diseases including malaria are known to consistently follow the “80/20 statistical distribution” [63] meaning that at least 80% of transmission occurs in 20% or less of all locations. This well established feature clearly implies that deliberately biasing the spatial distribution of any intervention to the most intense foci of vector density, which correspond to locations with higher than average encounter rates and therefore increased availability of the traps, will have correspondingly enhanced impacts upon malaria transmission.

In this model, spatially biasing the location of the traps based on this well-established phenomenon would effectively result in a four-fold enhancement of relative trap availability because with such deliberately biased trap placement, the rates of trap encounter are enhanced four times. Unlike in the case of unbiased placement, the relative trap availabilities (λ_i) are therefore enhanced not only by their longer attractive range, but also by the increased probability that the mosquitoes will encounter those extended odor plumes. It therefore follows that in a situation where these particular traps are biased to locations with 80% of all mosquitoes, their relative

availability increased a further four fold, which combined with the field estimates of the enhanced attractiveness yields relative availability of $\lambda_t = 4 \times 4 = 16$.

While targeted placement of traps to enhance availability might be achieved by mapping the relevant area and conducting geographic rather than household-based entomological surveys, sufficient resources and institutional capacity to accomplish this are not available in the vast majority of African communities. Nevertheless, we suggest that enough is known about mosquito distribution to enable informal selection of appropriate sites with a reasonable degree of accuracy in most settings that we are familiar with. The kinetic definition of availability, which we have formulated here implies that the availability of the traps for host-seeking mosquitoes will always be higher in areas close to aquatic habitats as this is where the mosquitoes emerge from and also where they return to lay eggs and restart their next host-seeking phase in the beginning of each feeding cycle [61,65,95,96]. Also, houses on the outskirts of aggregated human population such as towns and villages, or around breeding habitats within them [60-62,97,98] are always exposed to more mosquitoes than those in the centre because mosquitoes dispersing into such settlements inevitably feed predominantly on the hosts they encounter first which are, by definition, more available to them [96].

This quantitative and qualitative knowledge of mosquito dispersal processes suggests three alternative positioning strategies, which can be implemented even in the absence of fine-scale maps showing mosquito densities, and which can therefore also be used to achieve optimal targeting of the odor-baited traps (Figures 2A-C). Firstly, where the community is small, tightly aggregated and surrounded by numerous and dispersed aquatic habitats (particularly where these are cryptic or

unpredictably distributed) the best solution is probably to surround the perimeter of the settlement with traps (Figure 2A).

Secondly, where habitats are relatively few in number and easily identifiable, as may be the case in arid rural areas [99], surrounding the breeding sites may offer an even more effective strategy (Figure 2B). Urban areas where major areas of mosquito proliferation are usually surrounded by human settlement, rather than *vice versa* [97,98], represent a situation where these two strategies coalesce and are essentially equivalent (Figure 2C). It should therefore be possible, even without detailed maps of mosquito densities, to selectively position traps in ways that enhance their relative availabilities at least as well as the four-fold increase modelled here.

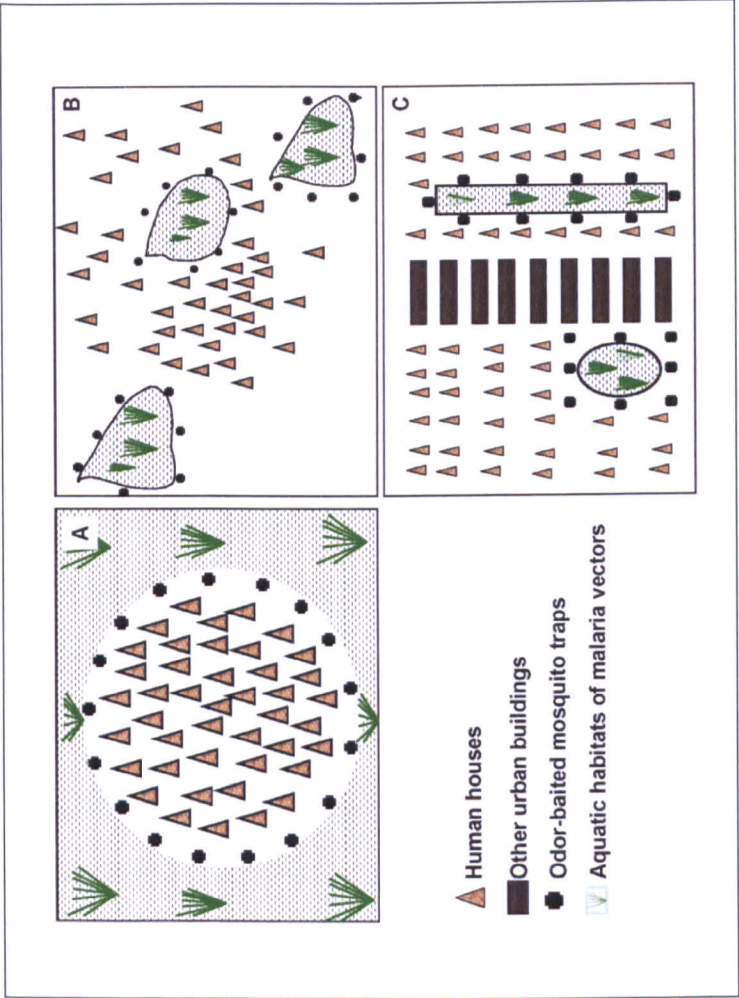


Figure 2: Alternative positional strategies for achieving optimal targeting of odor-baited mosquito traps. The figure shows places where the odor baited mosquito traps should be located in different scenarios namely: **A**; where the communities are small, tightly aggregated and surrounded by large or numerous aquatic habitats, **B**; where habitats are relatively few and easily identifiable such as in arid-rural areas and **C**; in urban settings where the main aquatic habitats are surrounded by human settlements. This diagram is not drawn to scale and is limited to basic structural representations of spatial relationships between human settlements and mosquito larval breeding sites.

Results

In all scenarios that we evaluated, odor-baited traps delivered useful levels of protection against malaria exposure with surprisingly few devices required per 1000 people, regardless of whether nets were in use or not (Figure 3). These simulations indicate that if the traps are baited with long range attractants that are at least four times as attractive to malaria mosquitoes as humans [41], and if they are located in areas where 80% of all mosquitoes are found [63], the traps on their own can confer community-wide protection equivalent to 50% coverage with ITNs.

The number of traps required to achieve these protection levels varies in different scenarios, ranging from 20 units to 130 units per thousand people (Figure 3). This rate translates to between 1 and 7 traps for every 50 persons, which assuming an average household size of 5, means that at optimum, a single trap would service up to 10 households. Figure 3 also shows that with a similarly modest number of efficient odor-baited traps, malaria transmission can be reduced by 99% or more in these hypothetical scenarios which are representative of most of sub-Saharan Africa. This is expected to occur more readily if the traps are used as complementary intervention alongside ITNs but is nevertheless also plausible if they are deployed as stand-alone vector control methods, especially in places where the primary vector is the anthropophagic *An. gambiae s.s.* (Figures 3A and C).

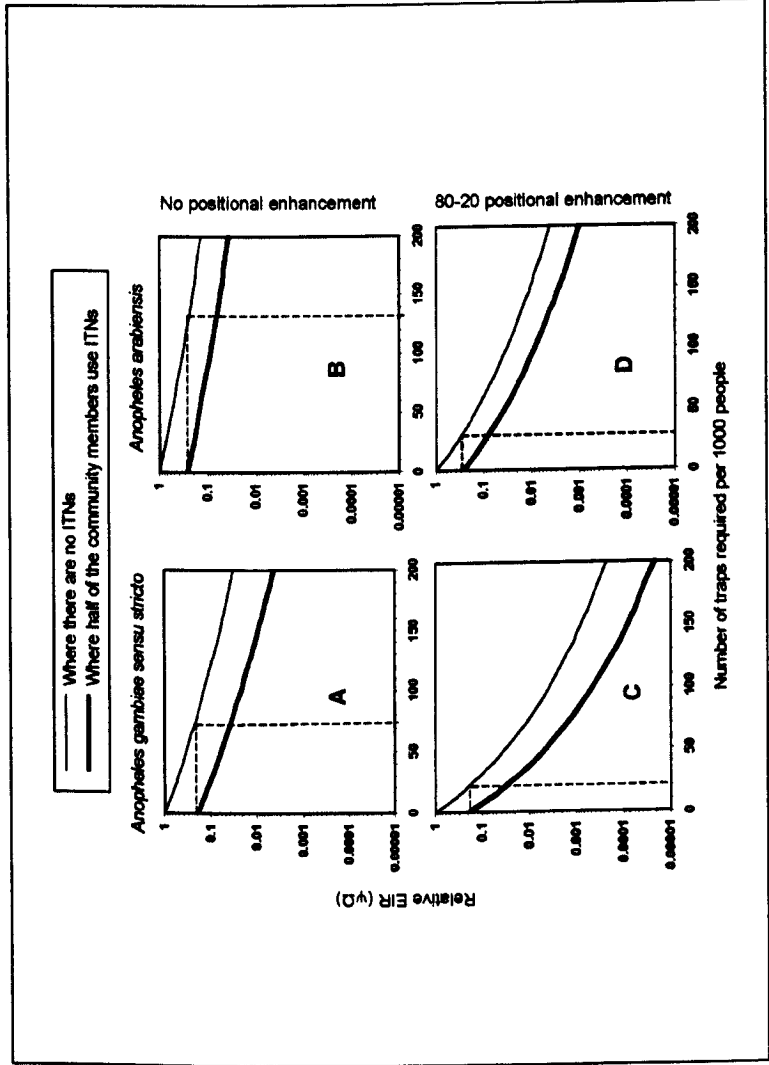


Figure 3: Effects of odor-baited mosquito traps on malaria transmission in situations with moderate ITN coverage. This figure depicts areas where: A; the primary vector is *Anopheles gambiae sensu stricto* and the trap locations are not spatially targeted, B; the primary vector is *Anopheles arabiensis* and the trap locations are not spatially targeted, C; the vector is *Anopheles gambiae sensu stricto* and the trap locations are spatially targeted to satisfy the 80-20 statistical distribution and D) where the vector is *Anopheles arabiensis* and trap locations are targeted to satisfy the 80-20 statistical distribution [63]. The dotted lines extrapolate the number of traps per 1000 people that would be required to achieve protection equivalent to ITNs if the traps are used alone. All simulated traps are baited with long-range odors that attract 4 times as many malaria mosquitoes as humans [41].

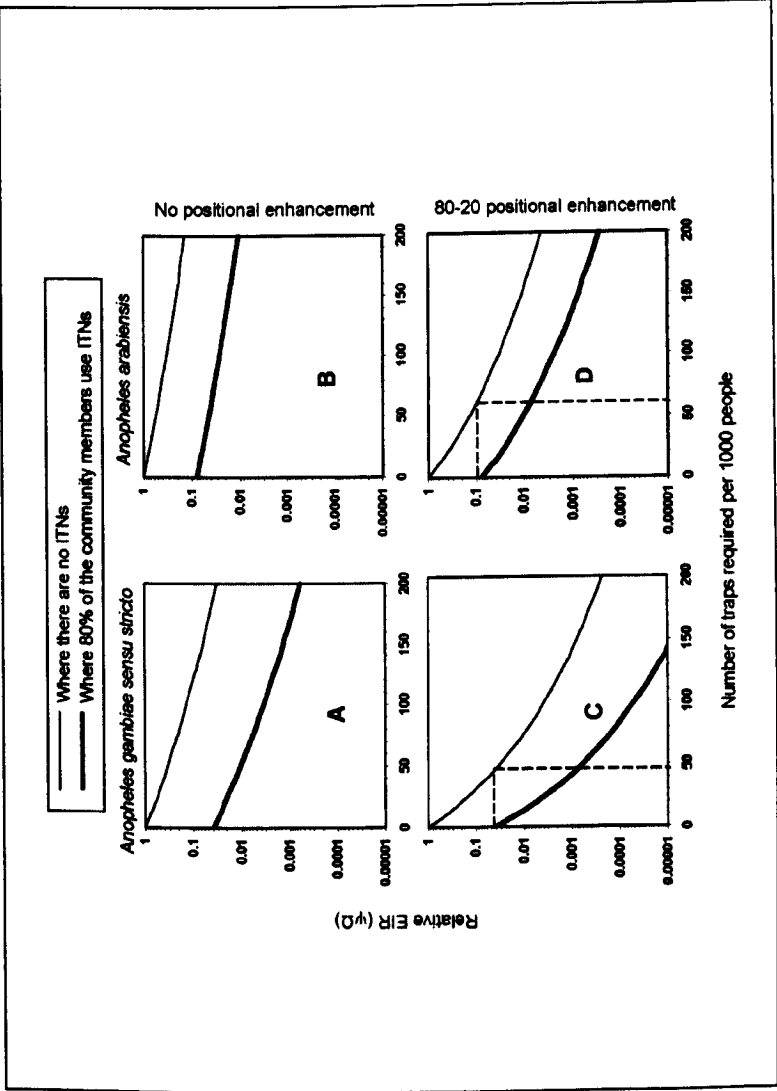


Figure 4: Effects of odor-baited mosquito traps on malaria transmission in situations with high ITN coverage. This figure shows that with high pre-existing ITN coverage (80% in this case), the combined intervention would yield far greater benefits with lower trap numbers than in situations with moderate ITN coverage (for example 50% shown in Figure 3). The dotted lines (not shown in panels A and B) extrapolate the number of traps per 1000 people that would be required to achieve protection equivalent to ITNs if the traps are used alone. All simulated traps are baited with long-range odors that attract 4 times as many malaria mosquitoes as humans [41].

Benefits of such combined interventions are likely to be greater where there is higher pre-existing ITN coverage. It is estimated that, in situations where 80% of community members use ITNs (Figure 4), malaria transmission could be reduced to far lower limits than in situations with 50% ITN coverage, even though the traps alone may not feasibly match the benefits of such high coverage with ITNs, without geographical targeting. For example, if we consider high transmission situations where unprotected persons are exposed to 200 infectious bites per person annually, 80% ITN coverage combined with about 45 traps per 1000 people could reduce relative exposure from 1 to 0.001, meaning an absolute reduction to 0.2 infectious bites per person per year (Figure 4).

Consistent with previous observations [38] and previous simulations of ITNs [43,44], malaria transmission by *An. arabiensis* in the presence of cattle can be more difficult to control than transmission in other scenarios because they readily feed upon the cattle, meaning that more vertebrate resources are available to these mosquito populations. Nevertheless, our simulations suggest that integrated vector management packages consisting of ITNs and odor-baited traps will still drastically reduce transmission in these situations. Figures 3B and 3D show that, so long as the availability of traps is enhanced by spatially targeted positioning, as few as 30 traps per 1000 people can achieve protection equivalent to 50% ITN coverage, even where such alternative hosts are available to the malaria vectors.

Benefits of odor-baited traps as a tool against malaria arise from their function as decoy hosts, which do not provide any blood but capture host-seeking mosquitoes that attack them. Figure 5 shows that malaria transmission is expected to decline drastically and exponentially in response to increases of the proportional contribution

of the traps, to the total availability of all hosts and pseudo-hosts that can be attacked by host-seeking malaria mosquitoes (C_A).

This term C_A , is best thought of as the coverage of all available host types with the trapping devices or the proportion of total host availability (A) that they account for. As the trap coverage (C_A) increases, EIR decreases dramatically and exponentially, regardless of the vector-host combinations or whether ITNs are used or not (Figure 5). The consistency of this trend across scenarios suggests that increasing individual trap availability by enhancing either the long-range attractiveness of these devices, increasing the number of traps, or by targeting the traps to the foci of highest mosquito density, is crucial to maximizing the epidemiological impact and/or minimizing the cost of this technology. It also elucidates a clear quantitative rationale for the attenuated impact of ITNs and traps upon vectors like *An. arabiensis*, which have alternative non-human hosts: such mosquito populations can exploit blood resources from a larger quantity of available hosts so a correspondingly greater quantity of traps are required to compete with the available natural hosts.

Lastly, as may be logically expected in nature, the simulations show that various mosquito feeding cycle processes and events that determine malaria transmission by the vector are reduced when odor baited traps are introduced, and when the number of traps is increased. For example, the feeding cycle length, the host seeking interval, and also the probability of surviving one complete feeding cycle, are all reduced (File S 6.1 provided on the CD accompanying this thesis).

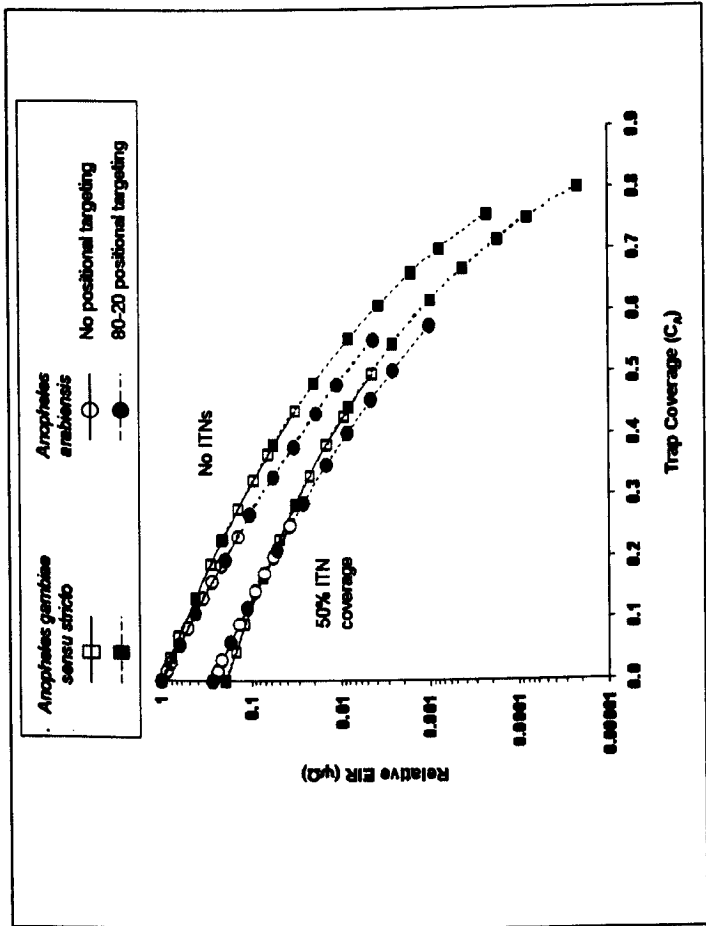


Figure 5: Relationship between trap coverage (C_A) and relative malaria exposure (Ψ_Ω). This figure shows predicted relationship between proportion of total availability of hosts and pseudo hosts that is accounted for by odor-baited traps (trap coverage; C_A) and resulting relative exposure to malaria (Ψ_Ω) when odor-baited mosquito traps are used in communities where there are no ITNs or in communities where half of the population already uses ITNs. The simulated traps are baited with long-range odors, which can attract at least four times as many malaria mosquitoes as humans [41]. The trap coverage (C_A) can be improved by several means, for example by increasing bait attractiveness, biasing trap locations towards areas with most mosquitoes, increasing the number of traps, or removing cattle from the area. Spatial targeting according to the 80-20 statistical distribution means concentrating the traps in areas where at least 80% of all mosquitoes are found [63]. All data points presented here are sampled from the simulations described in Figures 3 and 4.

Discussion

Using an adapted and conceptually reformulated mathematical model, we have successfully determined that odor-baited mosquito traps could potentially provide substantial protection against malaria risk in various epidemiological scenarios in sub-Saharan Africa. We have shown that even if existing coverage with insecticidal nets were 50%, traps could dramatically augment the benefits of ITNs. Although the simulated odor-baited mosquito traps can deliver encouraging levels of protection even when used on their own, the benefits are far greater when the traps are deployed to complement rather than to replace the ITNs (Figures. 3-5). This theoretical evidence reinforces the view that odor-baited traps could have genuine potential for malaria vector control [100,101] in Africa, where most of the present day malaria burden exists [78,91].

While this work encouragingly predicts that odor-baited traps might be developed into valuable tools for malaria transmission control, the simulated example is based on field evaluations of an experimental prototype [41], which would be prohibitively expensive for community-level scale-up or even large-scale efficacy trials. Improved, cost-effective trap models which translate such theoretical optimism into practical realization of malaria control therefore remain a future ambition to be pursued. While some progress has recently been made towards this goal [40,42], much remains to be done.

Perhaps the most useful outcome of this modeling exercise is therefore the identification of key characteristics that will determine the cost-effectiveness of these technologies, including how best they should be positioned and how best they may be delivered as a health commodity. First of all, the traps should be fitted with super-attractive odor lures, which can attract more mosquitoes than normal vertebrate hosts. Even though our

simulations considered traps baited with long-range lures that attract 4 times as many mosquitoes as humans, high trap coverage (C_A) values can be obtained even with baits that have lower degrees of attractiveness, so long as targeting of the traps to appropriate locations is proportionately enhanced by placing them in areas where mosquitoes are most abundant, or by simply using more traps. Developers of odor-baited trap technologies should therefore focus on odor baits that attract at least as many mosquitoes as real humans.

The other important characteristic is financial cost of the technology. If odor-baited traps were to be promoted for malaria control in Africa, they would need to at least match the cost-effectiveness of ITNs, which apart from being one of the primary interventions, are also one of the most cost-effective health commodities in existence, comparable with childhood vaccinations [102,103]. The most recent estimates based on 5 large-scale distribution programmes for insecticidal nets indicate it costs approximately US\$2.10 (Range 1.46 to 2.64) to provide one year of protection with a treated net [104].

Even assuming that each ITN is used by only one person so that 500 would be required to achieve 50% coverage of our simulated population of 1000, the 20 to 130 traps required to provide equivalent protection (Figure 3) would have to cost a maximum of 2005 US\$52.45 to \$8.07 per trap per year, respectively, to achieve equivalent cost effectiveness (File S 6.1). If we now consider that ITNs are commonly used by more than one person and adjust accordingly (mean of 1.9 occupants per net in the field setting where these trap prototypes were evaluated [105]), the standards of cost-effectiveness set by ITNs are even more challenging to match: Even if only 20 traps per 1000 people is sufficient, each would have to cost a maximum of US\$27.61 per annum for total costs of procurement, transport, installation, operation, maintenance while the less tractable *An. arabiensis* dominated

scenario requiring 130 traps per 1000 people indicates a maximum cost of \$4.25 per annum (File S 6.1).

Such low deployment costs are a lot to ask of any technology or implementation program and should be carefully considered by developers of odor-baited technologies for malaria transmission control. Developing a sufficiently cost-effective trap is probably the greatest technical hurdle this strategy must overcome to become a realistic option for malaria control programmes across Africa. Even if all the other necessary characteristics were fulfilled, developing devices which can affordably produce sufficient quantities of CO₂, the only bulk attractant in the current prototypes [41], is most probably the greatest challenge ahead. The experimental prototype of the odor-baited traps that we have considered here, as well as simpler more recent designs [40,42], remain far too expensive to consider at this stage for future large-scale use. In addition to the need for cheaper CO₂ generation, it also follows traps should be small and practical enough to be delivered and maintained in isolated African villages at reasonable costs.

Unlike ITNs which can be marketed as household consumer products, traps provide only communal benefits and would require a customized delivery mechanism to maximize its usefulness. We expect that even if the target product profiles that we have outlined here were manageable cost-wise, vertical and presumably community-based delivery mechanisms would be necessary to supply and deploy the traps. We propose that where local governance and administrative systems are already strengthened, or where they can be supported by centralized national malaria control programmes, sustainable implementation of a traps-based strategy may possibly be achieved through participatory approaches similar to those applied for scaling up community-based sanitation technologies like Ventilated Improved Pit (VIP)

latrines or water source protection among rural communities in developing countries [106-109].

We are not aware of any large scale malaria vector control operations which have used traps of any nature and with which we could directly compare our simulation results. Perhaps the most similar example is the 1980s tsetse fly control program in Zambezi valley, in Zimbabwe, where up to 3000 odor-baited tsetse fly targets treated with insecticides were deployed in an area of 600 square kilometres [14]. Considering the trap requirements predicted by our model, and comparing the simulated scenarios to this particular Zambezi valley tsetse fly program [14], it can be argued that traps might indeed be a viable option for further industrial development to combat malaria.

An obvious aspect of the outlined target product profile is that some of the essential trap characteristics can be traded off against each other. This is encouraging because such trade-offs may be undertaken to minimize costs of manufacture, installation or maintenance of the traps. For example, instead of super-attractive lures that may be too expensive to obtain, one may opt for moderately attractive lures but use larger numbers of more affordable traps and/or ensure that the trap positioning is enhanced.

None of these simulations would have been possible without reconsidering the fundamental biological definition of what an available host is and distinguishing this from the availability of blood. While host availability has been defined as either of these two possibilities (attackable hosts [52] versus blood [43,44] in previous models), this is the first time that this crucial distinction has been explicitly considered and separately parameterized. The combination of ITNs with odor-baited traps proved an ideal example because, while the former has a non-zero value for both parameters, traps provide no blood and cannot be plausibly represented with models which do not distinguish between these two quantities.

Beyond this specific application, this fundamental re-evaluation of how resource acquisition processes can be conceptualized may be particularly useful for modeling intervention options as diverse as mosquito repellents [110,111], house screening [112] and the auto-dissemination of larvicides [113] and slow acting adulticides [114].

Recent advances in mathematical modelling of how agricultural pests interact with pheromones suggest that such kinetic approaches could greatly improve evaluation of various interventions that use synthetic odor-cues, including not only host-derived attractants, but also pheromones usually used to disrupt insect mating in agricultural fields. For example in a recent publication by Miller *et al*, in which simple algebraic equations for attraction and competitive attraction were validated, *cumulative moth catches* were expressed as a function of *findability of trap baited with pheromone lures*, *efficiency of the traps*, the *retention time of the moths in the traps* and the *densities in an environment* [115]. If compared to the host-seeking processes of female mosquitoes as presented in this paper, *findability of traps* as presenter by Miller et al [115] may be considered analogous to *trap encounter rates* (Eq. 1 of this paper), while, *trap efficiencies* would be set to 1.0, with an infinite retention time of all mosquitoes that attack the traps, assuming that trapped mosquitoes do not escape afterwards. Nevertheless, it may be stated also that the current analyses deals more with competitive attraction, as opposed to non-competitive attraction, and that odor-baited mosquito traps must therefore have relative availabilities greater than 1.0, so as to be effective.

Though we consider these simulations to have been generally successful, we also recognize that there were some limitations with this particular model. For example, it is assumed that at the point when the vector attacks the host, there are only two possibilities: that either the vector feeds successfully and consequently survives or it dies in the attempt before obtaining a blood meal (Eq. 4). This argument implies that no mortality occurs after

blood meal acquisition, and instead considers all attack related mortality as occurring prior to feeding. This is not entirely true since there can be additional mortality immediately after feeding or midway through feeding, by which time malaria transmission may have occurred if the host was a susceptible human. As such, the model may slightly underestimate effects of ITNs on mosquito mortality. We therefore advise that our results be interpreted in view of protection from human exposure to infection as the model may not capture the full impact of ITNs on onward transmission, mediated by mosquitoes picking up parasites from a protected person and successfully transmitting the parasites to another person. Also, as has been the case with essentially all the deterministic malaria transmission models, with a few notable exceptions [51,96,116], our formulation does not consider fine scale spatial relations and heterogeneities in the dynamics of mosquito and human populations.

Lastly, it should be noted that in order for our findings to be generalizable to different transmission scenarios across Africa, this model formulation and also its previous versions [43, 44] use relative EIR on a log scale of 0 to 1 instead of empirical field estimates, to represent various outcomes of the modelled interventions. We recognize however, that for each individual scenario, it would be more reasonable to use absolute empirical indicators, such as mosquito trap catches, or malaria parasite prevalence rates. As such our simulations and findings do not exclude the essential need for field evaluation, by way of community scale trials, to ascertain the actual benefits of combining ITNs with odor-baited mosquito traps.

Nevertheless, these simulations do allow for much clearer quantitative insights into the future potential of odor-baited mosquito traps strategies for malaria transmission control.

Conclusions

Odor-baited mosquito traps could provide substantial protection against malaria in their own right and could augment benefits already achieved with ITNs if deployed as a complementary intervention. For this strategy to succeed, we propose that the following three key criteria should be met: 1) that the odor-baits should be considerably more attractive to malaria vectors than humans, 2) that the traps should be located in areas where host-seeking mosquitoes are concentrated and 3) that they need to be cheap and easy to deploy at a rate of 20-130 traps per 1000 people. Finally, if efficacious interventions matching this target product profile were developed, we recommend that the most appropriate way to deploy them effectively and sustainably would be through vertical rather than horizontal delivery mechanisms, which will require strong technical support from central authorities such as National Malaria Control Programmes, as well as broad progress towards improved governance and capacity of local authorities to implement such programmes on the ground.

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Conflicts of interest

None

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Chapter VII

Target product profile choices for intra-domiciliary malaria vector control pesticide products: repel or kill?*

Abstract

Background: The most common pesticide products for controlling malaria-transmitting mosquitoes combine two distinct modes of action: 1) conventional insecticidal activity which kills mosquitoes exposed to the pesticide and 2) deterrence of mosquitoes away from protected humans. While deterrence enhances personal or household protection of long-lasting insecticidal nets and indoor residual sprays, it may also attenuate or even reverse communal protection if it diverts mosquitoes to non-users rather than killing them outright.

Methods: A process-explicit model of malaria transmission is described which captures the sequential interaction between deterrent and toxic actions of vector control pesticides and accounts for the distinctive impacts of toxic activities which kill mosquitoes before or after they have fed upon the occupant of a covered house or sleeping space.

Results: Increasing deterrency increases personal protection but consistently reduces communal protection because deterrent sub-lethal exposure inevitably reduces the proportion subsequently exposed to higher lethal doses. If the high coverage targets of the World Health Organization are achieved, purely toxic products with no deterrence are predicted to generally provide superior protection to non-users and even users, especially where vectors feed exclusively on humans and

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a substantial amount of transmission occurs outdoors. Remarkably, this is even the case if that product confers no personal protection and only kills mosquitoes after they have fed.

Conclusions: Products with purely mosquito-toxic profiles may, therefore, be preferable for programmes with universal coverage targets, rather than those with equivalent toxicity but which also have higher deterrence. However, if purely mosquito-toxic products confer little personal protection because they do not deter mosquitoes and only kill them after they have fed, then they will require aggressive “catch up” campaigns, with behaviour change communication strategies that emphasize the communal nature of protection, to achieve high coverage rapidly.

Background

The most important front line vector control strategies for malaria prevention rely on killing mosquitoes that enter human houses by delivering insecticidal products to these domestic targets in the form of indoor residual spray (IRS) or long-lasting insecticidal nets (LLINs) [1, 2]. The common rationale underpinning these strategies is based on two well-established biological phenomena: 1) that the most important malaria vectors prefer to feed on humans and rest inside houses and 2) that a mosquito must feed several times on humans and, therefore repeatedly risk exposure to such insecticidal measures, before it is old enough to acquire, incubate and then transmit malaria to any human [3, 4]. As the most common and important species of *Plasmodium* that cause human malaria infections are strict anthroponoses, malaria vectorial capacity of a given mosquito species is directly and closely related to its human-feeding propensity so these two phenomena obviously co-occur in the most important vector populations [5].

This is particularly true in sub-Saharan Africa where, with some interesting exceptions, the bulk of human exposure to *Anopheles gambiae* and *Anopheles funestus* has occurred inside houses and these species feed almost exclusively upon humans [6-8]. As a result, even coverage of only half of the human population with LLINs or IRS can deliver huge reductions of transmission and substantial alleviation of malaria burden in settings where the challenge of eliminating malaria is greatest [9, 10]. Few public health interventions achieve such massive positive externality in the form of protecting those not directly covered [9-11] and the elegant way in which these measures exploit the biology of both the parasite and the vector is both intuitive and appealing [3, 4, 12]. The potential for community-level impact that is far greater than what can be achieved with personal protection alone is

obviously hugely attractive [2, 11, 12], but this simple rationale and recent progress with implementation masks a complex set of important product profile choices, which have thus far been made in the absence of decisive evidence or clear evaluation criteria.

However, the two most commonly used pesticides for controlling adult malaria vector mosquitoes, namely the synthetic pyrethroids and dichlorodiphenyltrichloroethane (DDT), combine two very distinct modes of action: 1) conventional toxicity which kills mosquitoes exposed to the pesticide while feeding or attempting to feed upon covered humans, 2) deterrence of mosquitoes away from those humans resulting from either irritation upon direct contact with the treated surface or even through spatial repellence from a distance of several meters [13-15]. Pyrethroids exhibit a strong combination of both contact irritant and spatial repellent properties, so that IRS and LLIN using these compounds often deter as many mosquitoes as they kill [16-20]. DDT is the only commonly used alternative to the pyrethroids for IRS and clearly has strong spatial repellency, as well as strong insecticidal effects upon mosquitoes that are not deterred and actually make contact [13, 14].

While high levels of deterrence enhance the personal protection afforded by a pesticide product and, therefore, uptake by the public, it may also attenuate or even reverse communal protection [15] because it diverts mosquitoes to non-users [21] rather than killing them outright. Theoretical analysis suggests that where vectors have a strict preference for human hosts, or their preferred alternative hosts are absent, such deterrent properties may be counterproductive or even dangerous [15]. In principle, diversion of mosquitoes away from protected individuals might cancel out the community-level benefits to non-users arising from decreased mosquito survival and infection rates and could even result in increased exposure because bites are increasingly focused on the unprotected people [15].

Numerous large scale field trials of insecticidal nets or IRS have produced overwhelming encouraging results [9, 10] but it is critical to note that these impacts result from products with a combination of deterrent and insecticidal properties. Even larger studies will be required to conclusively distinguish the community-level impacts of alternative profiles with deliberately formulated toxic versus deterrent product profiles. It is therefore unsurprising that there has been no such trial. While current guidelines for evaluating LLIN and IRS products in experimental huts [22] provide clear instructions on how to quantify personal protection and overall mortality rates of mosquitoes, it is not explicitly required to distinguish between toxic effects that kill mosquitoes before or after they feed and, with one exception [23], trials following these guidelines report only combined total mortality rates. Furthermore, consensus has yet to be attained regarding which of these evaluation criteria should be considered as primary and secondary or how the relative merits of these properties should be compared when evaluating existing products or designing new ones.

A process-based mathematical model of malaria transmission is outlined here, which captures the sequential interaction between deterrent and toxic actions of vector control pesticides and which accounts for the distinctive impacts of slow and fast-acting toxicity upon mosquitoes (Figure 1). This model is applied to explore how the interaction of deterrent and toxic actions affects both overall transmission intensity and its distribution across user and non-user groups in malarious communities. Furthermore, the consequent influence of alternative and hybrid product profiles upon the choice of optimal delivery system strategy is outlined and further potential applications for this model are discussed.

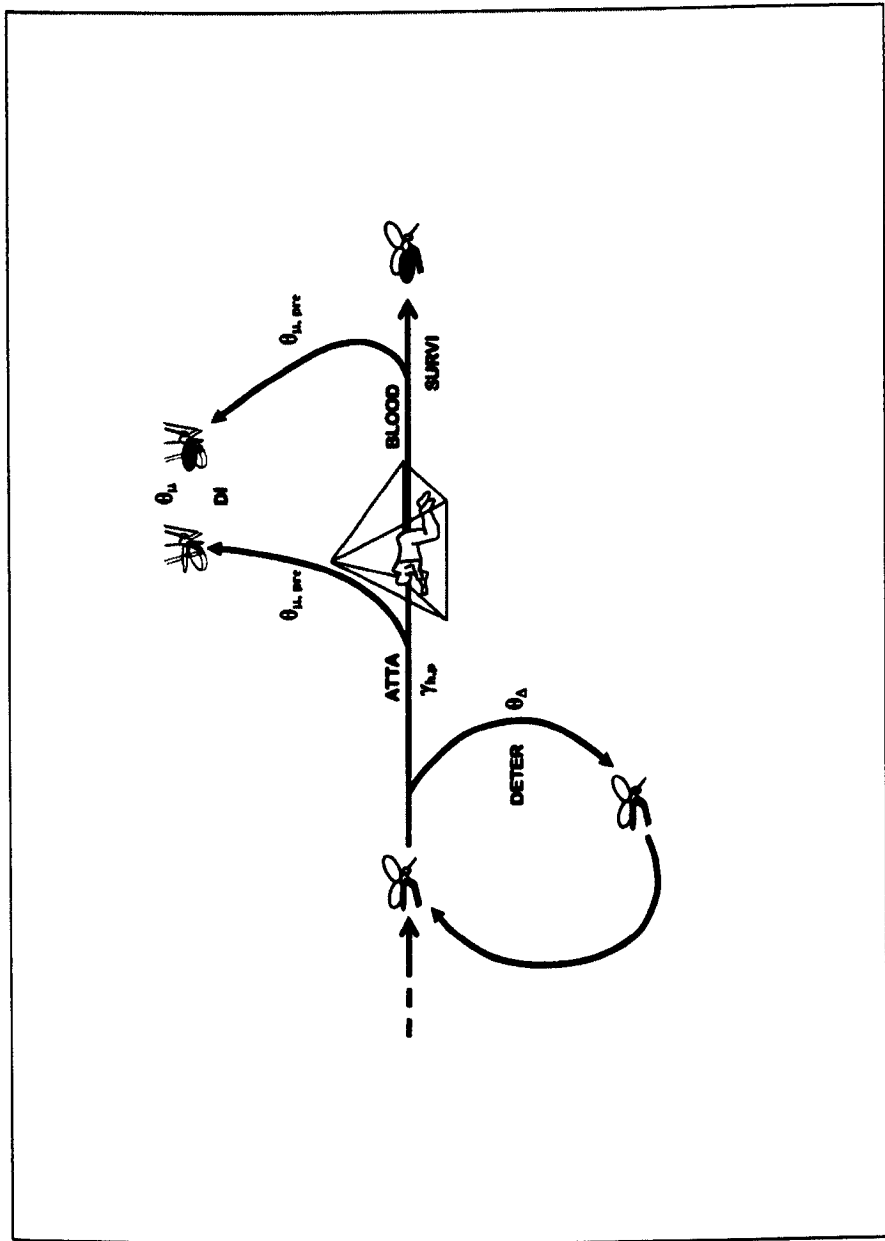


Figure 1. A schematic outline how the model captures the sequential nature of deterrent (θ_d) and toxic actions (θ_a) of vector control pesticides and account for the distinctive impacts of toxic activities which kill mosquitoes before ($\theta_{a,pre}$) or after ($\theta_{a,post}$) they have fed upon the occupant of a covered house (IRS) or sleeping space (LLIN).

Methods

Initially, a recently published deterministic model [24] was applied to elucidate how interactions between deterrent and insecticidal properties of hypothetical LLIN or IRS products might affect their impact upon malaria control when applied at high coverage across large populations. This exercise revealed that neither this formulation nor any of its predecessors [12, 15, 25] produced plausible, internally consistent outcomes for the probabilities of a mosquito attacking an encountered LLIN user and of successfully obtaining a blood meal when the proportion of human exposure that occurs at times when LLINs are used (π_t) was set to values less than 1.

The uncoupling of the impacts of π_t upon repellence and insecticidal activity became particularly obvious when the hypothetical LLIN was defined as being 100% repellent ($\theta_\Delta = 1$) and 100% insecticidal ($\theta_\mu = 1$): such simulations indicated that mosquitoes were directly killed by these nets, despite the expectation that coupled and complete repellency should prevent any such fatal contact. Furthermore, this implausible exposure of mosquitoes to direct mortality risk despite complete diversion away from such hazard increased as the proportion of exposure the LLIN can potentially prevent (π_t) decreased. Close examination of equations 6 and 7 of the original formulation [24] reveals how the previous approach caused the uncoupling of this conditionality to produce increasingly unrealistic outcomes as the fraction of exposure of indoor interventions for which the repellency does not apply ($1 - \pi_t$) increases, namely increasing estimated exposure of mosquitoes to the insecticidal activity and consequently nonsensically increasing insecticide-related mortality.

These flaws arise from inconsistent definition of protection, which was sometimes, but not always, considered to be synonymous with simply using a net. In simple terms, using

a net is something that covered individuals only do for approximately one third of a typical day so protection must be assumed to be partial, even for the most nocturnal, indoor-biting vectors, regardless of net efficacy [25]. Such interactions between mosquito and human behaviours are best summarized for indoor interventions such as LLINs or IRS in terms of the proportion of human exposure that would otherwise occur indoors (π_i) [25].

Published field estimates of this parameter for African malaria vector populations indicate that this proportion may fall far short of its optimal maximum value of 1 and may well be dropping in response to increasing selection pressure as ITN coverage increases [25-27]. Here these components of previously published formulations [12, 15, 24, 25] are harmonized so that this increasingly important *de facto* gap in coverage is treated with far greater clarity and internal consistency (See Table 1 for parameter definitions). In the interests of brevity and simplicity of language, the model description below refers consistently to an LLIN product but relates equally to an IRS product. Here, the essential changes to the existing model are described in detail and a brief but comprehensive description of the overall model is provided.

Coverage, protection and host availability to mosquitoes

Protection is defined as being conditional upon both using a net and, more specifically, using a net at times when transmission occurs [25]. The *de facto* protective coverage of humans ($C_{h,p}$) is therefore defined as being the product of crude coverage (C_h) and the proportion of human exposure that occurs indoors while asleep at times when LLINs are used (π_i) [25].

$$C_{h,p} = \pi_i C_h \quad (\text{Eq. 1})$$

The total availability for attack by mosquitoes [24] of protected ($A_{h,p}$) and unprotected humans ($A_{h,u}$) in the community is redefined so that individual users of nets exposed at times when they do not use them are considered to be unprotected. Thus, the effect of π_i upon host availability is applied as a conditional probability that affects population-level parameters in a coupled manner, rather than a probability which is independently applied to each of distinct individual-scale processes it influences in an uncoupled manner. The total availability of hosts protected against attack by using a net is therefore adjusted for this fraction of exposure which is directly preventable (π_i): The availability for attack of net users at times when those nets are used and therefore protect them is calculated as follows:

$$A_{h,net,p} = a_{h,p} N_h \pi_i C_h = a_{h,p} N_h C_{h,p} \quad (\text{Eq. 2})$$

Where $a_{h,p}$ is the availability for attack of an individual protected human, N_h is the number of humans and C_h is the crude coverage, estimated as the reported nightly usage rate.

The availability of the remaining fraction of humans which are unprotected ($A_{h,u}$) because either they do not use a net ($A_{h,o,u}$) or because they are exposed during times when the net is not used ($A_{h,net,u}$) can be calculated as follows where $a_{h,u}$ is the attack availability of an unprotected individual.

$$A_{h,u} = A_{h,o,u} + A_{h,net,u} = a_{h,u} N_h ((1 - C_h) + (1 - \pi_i) C_h) \quad (\text{Eq. 3})$$

Which can also be expressed simply as follows in manner consistent with equation 2:

$$A_{h,u} = a_{h,u} N_h (1 - \pi_i C_h) = a_{h,u} N_h (1 - C_{h,p}) \quad (\text{Eq. 4})$$

Similarly, to estimate the total availability of blood (Z) from these same categories of human hosts, equivalent formulae based on the availability of blood from individual protected ($z_{h,p}$) and unprotected ($z_{h,u}$) human hosts are applied:

$$Z_{h,net,p} = z_{h,p} N_h \pi_i C_h = z_{h,p} N_h C_{h,p} \quad (\text{Eq. 5})$$

$$Z_{h,u} = Z_{h,0,u} + Z_{h,net,u} = z_{h,u} N_h ((1 - C_h) + (1 - \pi_i) C_h) \quad (\text{Eq. 6})$$

$$Z_{h,u} = z_{h,u} N_h (1 - \pi_i C_h) = z_{h,u} N_h (1 - C_{h,p}) \quad (\text{Eq. 7})$$

By redefining protection and thus allowing for attenuated reductions of impact of insecticidal protection by human behaviours [25] at this population level the consistency and simplicity of parameters describing individual-level processes is improved. Individual mean ($a_{h,p}$ and $z_{h,p}$) and population total availability parameters ($A_{h,p}$ and $Z_{h,p}$) of the model are specified and calculated separately for protect and unprotected users and derived directly from the simpler respective un-weighted terms $\gamma_{h,p}$ and $\phi_{h,p}$, respectively. For diversion, this is achieved directly, similar to some previous formulations [12]:

$$\gamma_{h,p} = 1 - \Delta_{h,p} \quad (\text{Eq. 8})$$

Where $\Delta_{h,p}$ is the probability that a mosquito will divert away from an encountered, protected human host. However, the probability of feeding is expressed more explicitly than before, to consider only mortality which occurs before the mosquito feeds ($\mu_{h,p,pre}$) rather than total mortality ($\mu_{h,p}$) including those which feed but die soon afterwards:

$$\phi_{h,p} = \gamma_{h,p} (1 - \mu_{h,p,pre}) \quad (\text{Eq. 9})$$

Where $\mu_{h,p,pre}$ is the probability that a mosquito will die before feeding if it attacks a protected host. These terms are calculated as follows based on the probabilities of diversion ($\Delta_{h,u}$) and death ($\mu_{h,u}$) for unprotected humans, combined with the additional probability of diversion (θ_Δ) and death before feeding ($\theta_{\mu,pre}$) caused by the deterrent and insecticidal properties of the net:

$$\Delta_{h,p} = \Delta_{h,u} + \theta_\Delta (1 - \Delta_{h,u}) \quad (\text{Eq. 10})$$

$$\mu_{h,p,pre} = \mu_{h,u} + \theta_{\mu,pre} (1 - \mu_{h,u}) \quad (\text{Eq. 11})$$

This distinction, between toxic activities that act fast enough to prevent blood feeding and those that do not, necessitates that the total excess attack-related mosquito mortality resulting from using an LLIN (θ_{μ}) is specified as the sum of the excess mortality which occurs before ($\theta_{\mu,pre}$) or after ($\theta_{\mu,post}$) obtaining a blood meal:

$$\theta_{\mu} = \theta_{\mu,pre} + \theta_{\mu,post} \quad (\text{Eq. 12})$$

While insecticide-related mosquito mortality occurring after the mosquito has fed on the protected host does not contribute to personal protection, it does contribute to community-level suppression of malaria transmission by reducing population mean mosquito survival. The term $\mu_{h,p}$ is therefore calculated separately as follows:

$$\mu_{h,p} = \mu_{h,u} + \theta_{\mu} (1 - \mu_{h,u}) \quad (\text{Eq. 13})$$

This distinction between killing mosquitoes before or after feeding on the protected host allows the proportion of blood meals derived from humans (Q_h) to be calculated as previously described [24] based on this revised feeding probability term. Note, however, that this parameter therefore includes fatal blood meals obtained from insecticide-protected humans which mosquitoes never live long enough to digest. The meaning of parameters depending on the availabilities various categories of attackable hosts (A), rather than blood sources *per se* (Z) described above, such as the duration of the host-seeking interval (η_{ov}) and the probability of surviving host attack per feeding cycle (P_{γ}) [24] are unaffected. Note also that, as described below in equation 14, the latter logically remains based on $\mu_{h,p}$ rather than the new $\mu_{h,p,pre}$ term.

Table 1a: Definitions and explanations for symbols and abbreviations.

| Symbol | Definition and explanation |
|------------|--|
| a | Availability of individual hosts for attack: rate at which a single mosquito encounters and then attacks a given single host or pseudo-host [24]. |
| A | Total availability of hosts and pseudo hosts: rate at which a single mosquito encounters and attacks all hosts and pseudo hosts [24]. |
| b_h | The mean number of bites upon humans per emerging mosquito during its lifetime [15, 30]. |
| b | The mean number of bites upon all human and non-human hosts per emerging mosquito during its lifetime. |
| A_h | The mean number of infectious, sporozoite-infected bites upon humans per emerging mosquito during its lifetime [15, 30]. |
| A | The mean number of sporozoite-infected bites upon all hosts, regardless of their susceptibility to infection, per emerging mosquito during its lifetime. |
| c | Cattle [12, 15, 24, 28, 42]. |
| C_h | Crude coverage [12, 15, 24, 28, 42]: Proportion of people using LLIN as estimated in standardized malaria indicator surveys [82, 83]. |
| $C_{h,p}$ | Protective coverage: The proportion of all exposure of the human population which is effectively covered by LLIN use at times when that exposure actually occurs. |
| DDT | Dichloro-diphenyl-dicloroethylene [14]. |
| d | Probability that a mosquito which encounters a host will be diverted from that host [12, 15, 24]. |
| e | Host-encounter rate: rate at which a single host-seeking mosquito encounters a given single hosts [12, 15, 24, 28, 42]. |
| E | Emergence rate of mosquito vectors per year [12, 15, 24, 28]. |
| EIR | Entomological inoculation rate (mean number of infectious bites that an average individual human receives per year) [84-87]. |
| f | Probability that a mosquito which attacks a host will successfully feed upon that host [12, 15, 24, 28, 42]. |
| f | Feeding cycle length: measured as the number of days it takes a single mosquito to get from one blood feed to the next [12, 15, 24, 28]. |
| g | Gestation interval: number of days a mosquito takes to digest a blood meal and return to searching for oviposition site [12, 15, 24, 28]. |
| h or c | Humans or cattle, respectively [12, 15, 24, 28, 42]. |
| IRS | Indoor residual spraying [10, 49] |
| κ | Human infectiousness to mosquitoes: probability of a vector becoming infected per human bite [29, 30, 88, 89]. |
| LLIN | Long-lasting insecticidal net [90] |
| λ | Relative availability for attack of a given non-human host type, calculated as quotient of the mean individual attack availability of those hosts divided by the mean individual attack availability of humans not using LLINs [24]. |
| μ | Probability that a mosquito which attacks a host will die during the attack [12, 15, 24]. |

Table 1b (continued from Table 1a)

| Symbol | Definition and explanation |
|---------------------------------|--|
| η_o | Oviposition site-seeking interval: number of days a mosquito takes to find an oviposition site once it starts searching for it [12, 15, 24, 28]. |
| η_v | Host-seeking interval: number of days a mosquito takes to find and attack a vertebrate host [12, 15, 24, 28]. |
| net or 0 | LLIN user or non-user, respectively |
| N | Number of hosts [12, 15, 24, 28]. |
| θ_A | Excess proportion of mosquitoes which are diverted while attempting to attack a human while using an LLIN [24]. |
| θ_k | Excess proportion of mosquitoes which are killed while attacking a human while that person is using an LLIN [24]. |
| $\theta_{k,pre}$ | Excess proportion of mosquitoes which are killed before blood feeding while attacking a human while using an LLIN. |
| $\theta_{k,post}$ | Excess proportion of mosquitoes which are killed after blood feeding while attacking a human while that person is using an LLIN. |
| n or 0 | Intervention package scenarios consisting of a specific coverage with LLINs with specific deterrent and toxic properties, with 0 denoting baseline conditions with negligible net coverage, simulated by setting $c_k=0.001$ [24]. |
| π_i | The proportion of normal exposure to mosquito bites upon humans lacking LLINs, which occurs indoors at times when nets would normally be in use [25-27, 37]. |
| p or u | Specifies values of parameters for humans while actually using and protected by an LLIN, or those which are unprotected who do not use or are outside of their nets, respectively. |
| P | Probability that a resting mosquito survives any one day [15, 91]. |
| P_f | Probability that a mosquito survives a single complete feeding cycle [12, 15, 24, 28, 30]. |
| P_{ov} | Probability that a mosquito survives any full day of the oviposition site-seeking interval or host-seeking interval [12, 15, 24]. |
| Q_h | Human blood index: the proportion of all blood meals from all hosts which are obtained from humans [12, 15, 24, 28, 30]. |
| γ | Probability that a mosquito attacks an encountered host [12, 15, 24]. |
| ψ | Relative exposure of different hosts other than unprotected humans to infectious mosquito bites: calculated as a ratio of exposure of those hosts to exposure of humans not using nets [24]. |
| WHO | World Health Organization |
| λ | Availability of blood from an individual host: rate at which a single mosquito encounters, attacks and successfully feeds upon a given single host [24] |
| $\lambda, \lambda_h, \lambda_c$ | Total availability of blood from all hosts, all humans and all cattle, respectively: rate at which a single mosquito encounters, attacks and successfully feeds upon these host sets [24] |
| λ_a | Total availability of aquatic habitats: rate at which a single mosquito encounters and successfully oviposits into all aquatic habitats |

Implications of redefining coverage, protection and host availability for mosquito population parameters

Previous versions of this model incorporated the lack of an effect of an LLIN on outdoor malaria transmission π_t by either treating it as a weighting term for calculating population mean values for feeding probability and attack-related mortality [12, 25] or by applying directly to the individual level diversion and mortality processes [15, 24]. The changed manner in which protection, coverage and availability are conceptually distributed (equations 1 to 7 and associated text), means that population-level parameters such as the proportion of blood meals obtained from humans (Q_h) and mean host-seeking interval (η_v), can all be simply calculated in terms of total host attack (A) and blood (Z) availability parameters exactly as previously described [15, 24]. Note, however, that this means that the published breakdowns of these population-level parameters into functions of the products of numbers of hosts (N) and mean individual availabilities (a and z , respectively) [15, 24] are no longer valid.

For other population-level parameters, simpler, more direct and intuitively satisfying derivations are implied. For example, this approach allows ready estimation of the probability of surviving host attack per feeding cycle (P_y) based on the mosquito mortality rates (μ) and corresponding community-wide total attack availabilities (A) of protected humans (h,p), unprotected humans (h,u) and cattle (c).

$$P_y = 1 - \left(\frac{\mu_{h,p} A_{h,p} + \mu_{h,u} A_{h,u} + \mu_c A_c}{A_{h,p} + A_{h,u} + A_c} \right) \quad (\text{Eq. 14})$$

Otherwise, all the mosquito population parameters are calculated exactly as previously described, and outlined as follows.

The mean seeking interval for vertebrate hosts (η_v) can be calculated as the reciprocal of total host availability (A), using estimates of these feeding probabilities and their corresponding encounter rates [24, 28]:

$$\eta_v = \frac{1}{A} = \frac{1}{A_{hu} + A_{hp} + A_c} \quad (\text{Eq. 15})$$

The feeding cycle length (g) is calculated as the sum of the durations of the gestation period (g), the oviposition site-seeking interval (η_o) and the vertebrate host-seeking interval (η_v):

$$f = g + \eta_o + \eta_v \quad (\text{Eq. 16})$$

Survival across all phases of the gonotrophic cycle is calculated as the distinct daily survival probability during each phase to the power of the respective time intervals, namely the host-seeking interval (η_v), gestation period (g) and oviposition site-seeking interval (η_o). The daily survival probability of a resting mosquito is defined as P and the survival probabilities during host-seeking and oviposition site-seeking are assumed to be equal and are both defined using the term P_{ov} . The survival rate per feeding cycle (P_f) was estimated as the combined probability that a vector survives gestation (P^g), oviposition site-seeking ($P_{ov}^{\eta_o}$), vertebrate host-seeking ($P_{ov}^{\eta_v}$) and the eventual attack of a host ($P_{ov}^{\eta_v + \eta_o}$):

$$P_f = P^g P_{ov}^{\eta_v} P_{ov}^{\eta_o} P = P^g P_{ov}^{\eta_o + \eta_v} P \quad (\text{Eq. 17})$$

Similarly, the human blood index is calculated as the proportion of total blood availability accounted for by humans [24]:

$$Q_h = \frac{Z_{hu} + Z_{hp}}{Z_{hu} + Z_{hp} + Z_c} \quad (\text{Eq. 18})$$

The biodemography component of the model is adapted to a daily cycle and cumulative survival up to each age (x) is estimated as follows [15]:

$$P_x = P_f^{x/f} \quad (\text{Eq. 19})$$

Similarly, the sporozoite infection prevalence of mosquitoes at each age is considered in days, accounting for superinfection:

$$S_x = S_{x-1} + \frac{\kappa Q_h(1-S_{x-1})}{f} \text{ where } x > n \text{ otherwise } S_x = 0 \quad (\text{Eq. 20})$$

where κ denotes the mean infectiousness of the human population to vector mosquitoes [29] and n is the duration of the sporogonic development period of the parasite from ingestion to infective sporozoite stages [30]. Survival and infectiveness probabilities are calculated up to 40 days, after which the contributions of mosquitoes in these age classes to transmission become negligible. Note that P_x is multiplied by S_x to obtain the corresponding probability of being both alive and infective (I_x) on each day

The following mosquito lifetime biodemographic parameters are calculated by summing these three age-specific outcomes as previously described [15, 30]. The number of human bites the average mosquito takes in a lifetime (b_h) is defined as the sum of the probabilities of surviving and feeding on a human at each age (x):

$$b_h = \frac{Q_h}{f} \sum_x^\infty P_x \quad (\text{Eq. 21})$$

Note that to enable incorporation of survival-dependent emergence rates, the number of human bites on all hosts, rather than just humans, per mosquito lifetime (b) is similarly calculated:

$$b = \frac{1}{f} \sum_x^\infty P_x \quad (\text{Eq. 22})$$

Accounting for superinfection, the number of infectious bites on humans per mosquito lifetime (β_h) is calculated as the product of the human blood index and sum of the products of the probabilities of biting and being infectious at each age [15, 30]:

$$\beta_h = \frac{Q_h}{f} \sum_x^\infty S_x P_x \quad (\text{Eq. 23})$$

Again, the number of sporozoite-infected bites on all hosts per mosquito lifetime (β), regardless of whether that host is susceptible to infection or not, is calculated similarly but ignoring the human blood index term:

$$\beta = \frac{1}{f} \sum_x^{\infty} S_x P_x \quad (\text{Eq. 24})$$

The overall sporozoite prevalence in the vector population (S) can then be calculated as β_h divided by b_h :

$$S = \beta_h / b_h = \beta / b \quad (\text{Eq. 25})$$

Epidemiological outcomes: dealing with partially covered, partially protected humans

Also, the entomologic inoculation rate (EIR) for non-users ($EIR_{h,0}$) can be directly estimated based on the share of all available blood sources which a single non-user represents ($z_{h,u}/Z$) multiplied by the total number of infectious bites on all hosts (β ; equation 24) by all emerging mosquitoes (E):

$$EIR_{h,0} = \frac{z_{h,u} \beta E}{Z} \quad (\text{Eq. 26})$$

Alternatively, this parameter may be estimated by considering only infectious bites on human hosts (β_h ; equation 23) and therefore considering only the share of available human blood which such an individual represents:

$$EIR_{h,0} = \frac{z_{h,u} \beta_h E}{z_h} = \frac{z_{h,u} \beta_h E}{z_{h,0} + z_{h,net}} \quad (\text{Eq. 27})$$

Nevertheless, it is essential to retain the protection-weighted mean terms for parameters which reflect the properties of individual net users who are only covered with the protective LLIN for proportion of their normal exposure (π_i) and uncovered and unprotected for the remained ($1 - \pi_i$). These terms are therefore retained but annotated more distinctly than

previously [12] so that the attack probability ($\gamma_{h,net}$ rather than $\bar{\gamma}_{h,p}$) and feeding probability ($\phi_{h,net}$ rather than $\bar{\phi}_{h,p}$) reflect the mean of protected and unprotected periods for net users, but cannot be confused with the corresponding probabilities for net users during the specific periods when they are protected ($\gamma_{h,p}$ and $\phi_{h,p}$, respectively).

$$\gamma_{h,net} = \pi_i \gamma_{h,p} + (1 - \pi_i) \gamma_{h,u} \quad (\text{Eq. 28})$$

$$\phi_{h,net} = \pi_i \phi_{h,p} + (1 - \pi_i) \phi_{h,u} \quad (\text{Eq. 29})$$

Consequently, derived terms such as attack availability ($a_{h,net}$ rather than $\bar{a}_{h,p}$) and blood availability ($z_{h,net}$ rather than $\bar{z}_{h,p}$), as well as corresponding terms for relative attack availability ($\lambda_{h,net}$ rather than $\lambda_{h,p}$) and exposure to bites ($\psi_{h,net}$ rather than $\psi_{h,p}$) compared with non-users, can be calculated as previously described.

$$a_{h,net} = \varepsilon_h \gamma_{h,net} \quad (\text{Eq. 30})$$

$$z_{h,net} = \varepsilon_h \phi_{h,net} \quad (\text{Eq. 31})$$

$$\lambda_{h,net} = \frac{a_{h,net}}{a_{h,o}} \quad (\text{Eq. 32})$$

$$\psi_{h,net} = \frac{z_{h,net}}{z_{h,o}} \quad (\text{Eq. 33})$$

Consequently, the EIR experienced by net users can be calculated by five different but consistent means:

$$EIR_{h,net} = \frac{z_{h,net} \beta_R}{z} = \frac{z_{h,net} \beta_h R}{z_h} = \frac{z_{h,net} \beta_h R}{z_{h,o} + z_{h,net}} = \frac{z_{h,net} \beta_h R}{z_{h,u} + z_{h,net,p}} = \psi_{h,net} EIR_{h,o} \quad (\text{Eq. 34})$$

Additionally, the mean EIR experienced in scenario Ω by the mixture of net users and non-users which comprise the community ($\psi_{h,\Omega}$) can be independently calculated in three distinct ways which yield consistent results. Consistent with equation 22 of Okumu *et al.* [24], this parameter can be estimated by simply weighting the EIR parameters for net users and non-users according to crude coverage and the gap in coverage, respectively:

$$EIR_{h,\Omega} = C_h EIR_{h,net,\Omega} + (1 - C_h) EIR_{h,o,\Omega} \quad (\text{Eq. 35})$$

However, it is also possible to calculate exactly the same values with a simpler formula derived from first principles, based on the assumptions of the very first of this family of models [30]:

$$EIR_{h,\Omega} = \frac{\beta_h \mathcal{E}}{N_h} \quad (\text{Eq. 36})$$

Reassuringly, identical values can also be calculated as described above by weighting the availability of blood from protected and unprotected individuals according to *de facto* protective coverage ($C_{h,p}$) rather than crude coverage (C_h).

$$EIR_{h,\Omega} = \frac{(C_{h,p} z_{h,p} + (1 - C_{h,p}) z_{h,u}) \beta_h \mathcal{E}}{z_{h,o} + z_{h,net}} = \frac{(C_{h,p} z_{h,p} + (1 - C_{h,p}) z_{h,u}) \beta \mathcal{E}}{z} \quad (\text{Eq. 37})$$

Similarly, the relative exposure of non-users and users of nets ($\psi_{h,o,\Omega}$ and $\psi_{h,net,\Omega}$ rather than $\psi_{h,p,\Omega}$ and $\psi_{h,u,\Omega}$, respectively) and community-wide mean relative exposure ($\psi_{h,\Omega}$) in a given intervention scenario (Ω) is calculated exactly as previously described except that the terms $EIR_{h,o,0}$, $EIR_{h,o,\Omega}$ and $EIR_{h,net,\Omega}$ replace $EIR_{h,u,0}$, $EIR_{h,u,\Omega}$ and $EIR_{h,p,\Omega}$ to denote the EIR experienced by non-users in a scenario with no intervention and that of non-users and users under intervention scenario Ω , respectively:

$$\psi_{h,o,\Omega} = \frac{EIR_{h,o,\Omega}}{EIR_{h,o,0}}$$

(Eq. 38)

$$\psi_{h,net,\Omega} = \frac{EIR_{h,net,\Omega}}{EIR_{h,o,0}} \quad (\text{Eq. 39})$$

$$\psi_{h,\Omega} = \frac{EIR_{h,\Omega}}{EIR_{h,o,0}} \quad (\text{Eq. 40})$$

Survival-dependent mosquito proliferation

Previous formulations of this model have assumed that larval habitats are always at their carrying capacity so the annual emergence rate of mosquitoes (E) is fixed, regardless of vector survival rates. In reality, vector populations experience dramatic seasonal fluctuations in larval habitat availability so while this assumption is largely true during drier times of the year when the quantity of habitat is static or contracting, it is rarely limiting during the onset or peak of the rains when vector populations can grow at their maximum reproduction rate [31, 32]. Furthermore, observations of the differential impact of insecticide-treated nets upon sibling species composition within the *An. gambiae* complex [33, 34] and impact of indoor-residual spraying upon inter-species competition within the *An. funestus* group [35, 36], both confirm that oviposition input into larval habitats does limit vector population sizes. These simulations were, therefore, executed both with and without allowing for adult survival-dependent emergence rates which were calculated as follows.

Emergence rate was assumed to vary simply and linearly with mean number of successfully-completed feeding cycles by adult mosquitoes (b ; Equation 22). Emergence rate in a given vector control scenario (E_{Ω}) was therefore calculated as the product of the maximum emergence rate expected in the absence of any adult mosquito control (E_0) and the relative value of the mean number of feeding cycles per mosquito lifetime in that scenario (b_{Ω}), compared with such baseline conditions (b_0):

$$E_{\Omega} = E_0 b_{\Omega} / b_0 \quad (\text{Eq. 41})$$

The calculations for the feeding cycle duration itself (f) as the sum of the gestation (g), oviposition site-seeking (η_o) and vertebrate blood-seeking (η_v) intervals are exactly as previously described [15]:

$$f = g + \eta_o + \eta_v \quad (\text{Eq. 42})$$

Consistent with the previously published definition of host availability [24], it is assumed that protecting hosts does not alter their location, or the rate at which they are encountered by kinesis, but rather extends the spatial distribution of locations to which mosquitoes must disperse to in order to obtain blood. As hosts are increasingly protected, a greater mean number of hosts must be encountered before a blood meal can be successfully obtained. Longer host-seeking intervals, that include a greater mean number of unsuccessful host encounters, will inevitably result in a mean increase in the distance and duration of subsequent return journeys to oviposition sites. Calculation of the oviposition site-seeking interval (η_o) is adapted to account for the expectation that mosquitoes forced to fly further and longer in search of blood will also have to fly proportionally further and longer in search of oviposition sites once the blood meal has been digested and eggs are matured. This term is calculated as the reciprocal of aquatic habitat availability, termed Z_a rather than A_a , as previously described [28], to maintain consistency with the separate definitions of rates of initiation and completion of resource utilization processes here and elsewhere [24]:

$$\eta_o = 1/Z_a \quad (\text{Eq. 43})$$

However, here this term (Z_a) is assumed to vary proportionally with vertebrate blood availability (Z) as it changes from baseline (0) to intervention (Ω) scenarios, reflecting the intrinsically endogenous relationship between host and aquatic habitat availability:

$$Z_{a,\Omega} = Z_{a,0} Z_{\Omega}/Z_0 \quad (\text{Eq. 44})$$

Parameterization of the model

The parameters of the model were set exactly as previously described [24] with the following adaptations, all of which are summarized in Table 2. The term π_i is set at a values of 0.90, consistent with published reports from areas with high coverage of untreated nets [25, 37] and historical field observations for African vector populations from across Africa (Huho *et al.*, Unpublished) or at 0.50, reflecting more recent observations from vector populations exposed to high coverage of LLINs, IRS or house screening [25-27, 38].

Previous modelling investigations [15, 39] have illustrated that the eventual impact of deterrent pesticide products upon malaria transmission exposure for non-users is very sensitive to the assumed value for mosquito survival while foraging for vertebrate blood or oviposition site resources (P_{ov}), parameter for which no field estimates exist to the authors knowledge. A range of values of were examined in the absence of any intervention measure ($C_h = 0$) to determine an approximate value that is most compatible with the observed biodemographic profiles of real populations of vectors and sporogonic parasites in the field. Implausibly low values for the proportion of mosquitoes surviving each feeding cycle (P_f) except at high assumed values of P_{ov} , approaching the likely upper limit of 0.90 defined by the estimated survival rate of resting mosquitoes (Figure 2).

Furthermore, surprisingly low sporozoite prevalence (S) rates were predicted for both species, especially at the lower end of the range of assumed P_{ov} , suggesting that values of the latter are high in nature. However, actual field estimates for survival per feeding cycle ($P_f = 0.62$) and sporozoite prevalence ($S = 0.016$) for the village of Namwawala in the 1990s, where the crucial human population size (N_h) and availability parameters (a_h) were obtained from, were quite low by the standards African vector populations in the absence of LLINs or

IRS and compare reasonably well with the *An. gambiae* scenario simulated here where $P_{ov} > 0.85$. Note that although transmission in this village was dominated by *An. arabiensis* at this time, no significant cattle population existed so the *An. gambiae* scenario assuming no alternative hosts is most representative of this setting. While daily survival rates for actively foraging mosquitoes (P_{ov}) must be somewhat lower than for resting mosquitoes, normal parity and sporozoite rates of African vector populations can only be plausibly explained if this difference is small, so P_{ov} was set at 0.85 for all subsequent simulations.

All other parameter settings for the two vector population scenarios (*An. arabiensis* representing a mosquito that can exploit non-human hosts compared with *An. gambiae* which is almost exclusively dependent on humans for blood) are as previously described for a village with 1,000 people and an equal number of cattle [24].

Specifically, the mean individual attack availability of unprotected humans ($a_{h,u}$) to *An. arabiensis* in this particular Tanzanian village in the 1990s was calculated as the reciprocal of the estimate of the mean vertebrate host-seeking interval (η_v), based on the distribution of ovariolar stalks dilation status among host-seeking specimens [40], divided by the number of humans present at the time [24, 28]. This approach to estimating this parameter was first described [28] before clear distinction between the availability of individual hosts for attack ($a_{h,u}$) and the availability of individual blood sources *per se* ($z_{h,u}$) had been explicitly outlined [24] but is even more appropriate when the former is specified.

The same $a_{h,u}$ value of 1.2×10^{-3} attacks per host per night per host-seeking mosquito was assumed for *An. gambiae*. The mean individual attack availability of cattle (a_c) for each species was calculated by multiplying the equivalent parameter for humans ($a_{h,u}$) by field estimates [41] of the relative availability of cattle blood, compared to that of humans (ψ_c), for

both vector species [42], yielding estimates of 1.9×10^{-3} and 2.5×10^{-5} attacks per host per night per host-seeking mosquito, for *An. arabiensis* and *An. gambiae*, respectively. Note that this calculation assumes that for unprotected hosts, the probability of successfully feeding upon an attacked host is equivalent for the two host types ($\mu_{h,u} = \mu_c$) so that the relative availability of cattle for attack is equivalent to the relative availability of cattle blood ($\lambda_c = \psi_c$).

Consistent with previous simulations, the maximum emergence rate of mosquitoes in the absence of adult mosquito control measures (E_0) was set at 2×10^7 adult mosquitoes per year. Except where stated otherwise, crude coverage of humans was set at 80% ($C_h = 0.8$) in line with the Roll Back Malaria targets for coverage of all age groups with LLINs which represents an ambitious but realistically achievable target for most malaria afflicted developing nations.

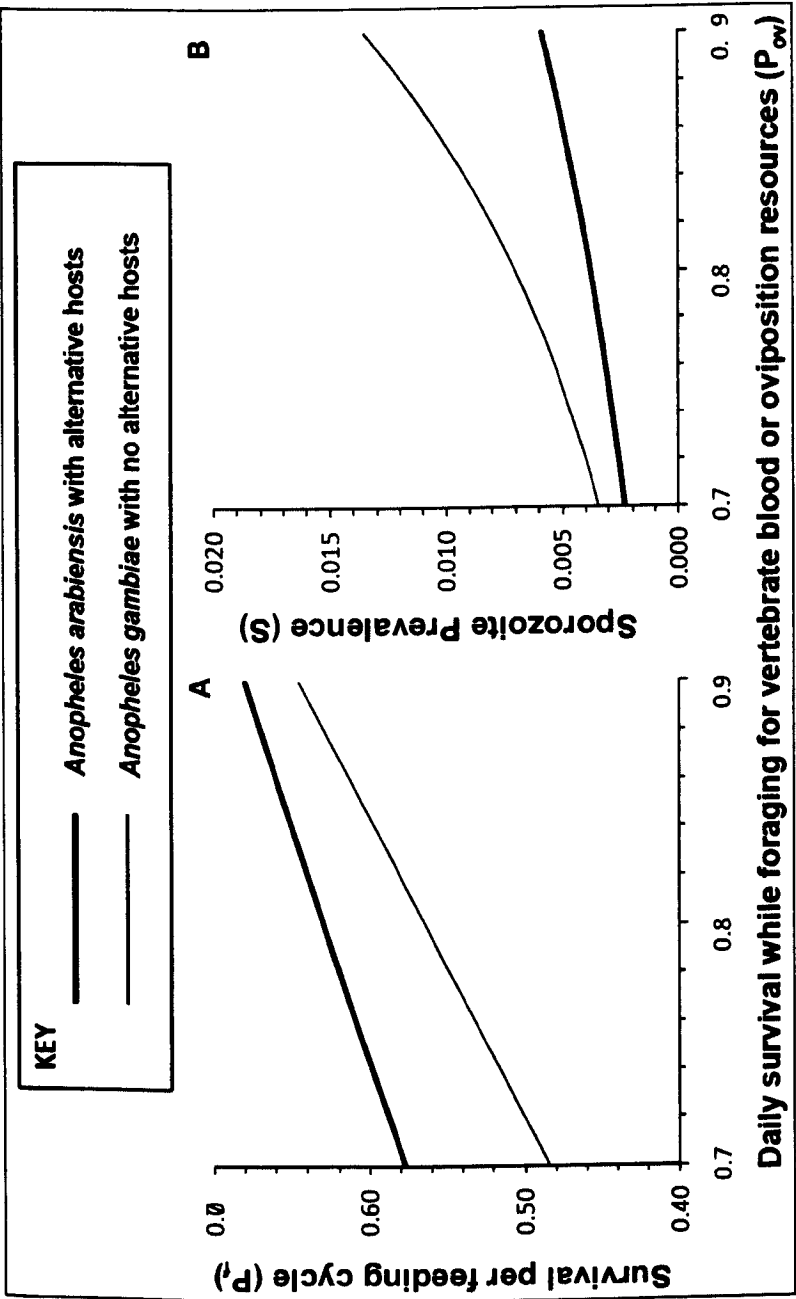


Figure 2. The sensitivity of mosquito survival per feeding cycle (P_f) and sporozoite infection prevalence (S) upon assumed values for daily survival while foraging for vertebrate blood or oviposition resources (P_{ov}) in the absence of any LLIN or IRS intervention ($C_h = 0$).

Table 2: Values and references for ecological parameters in the simulations

| Definition | Symbol | Value | References |
|--|----------------|----------------------|------------------------------|
| Total number of cattle | N_c | 1000 | [15] |
| Total number of humans | N_h | 1000 | [92] |
| Diversion probability from an unprotected vertebrate host (cattle or human) | $\Delta_{h,u}$ | 0.1 | [93] |
| Mortality probability upon attacking an unprotected host | $\mu_{h,u}$ | 0.1 | [93] |
| Mean availability of individual unprotected humans ^a | $a_{h,u}$ | 1.2×10^{-3} | [28, 40] |
| Mean availability of individual cattle ^b | a_c | | |
| <i>An. arabiensis</i> | | 1.9×10^{-3} | [28] |
| <i>An. gambiae s.s.</i> | | 2.5×10^{-5} | [28, 42] |
| Total availability of aquatic habitats | Z_a | 3 | [28] |
| Duration of gestation | g | 2 | |
| Proportion of mosquitoes surviving per day while feeding while resting | P | 0.9 | [91] |
| Proportion of mosquitoes surviving per day while foraging for hosts or oviposition sites | P_{ov} | 0.85 | Figure 2 and associated text |
| Duration of the parasite sporogonic development period | n | 11 | [30] |
| Human infectiousness to mosquitoes | κ | 0.03 | [29] |
| Total number of adult mosquitoes emerging per year | E | 2.0×10^7 | [24] |

^a The value of the parameter is equivalent to attacks per day per host-seeking vector per unprotected human.

^b The value of the parameter is equivalent to attacks per day per host-seeking vector per individual head of cattle and was different for the two vector species *Anopheles arabiensis* and *Anopheles gambiae sensu stricto*. With the exception of this parameter, all the other values are assumed to be identical for both species.

Results

The fundamental trade-off between toxic and deterrent actions (Figure 1) is clearly illustrated by the simulation results presented in Figure 3, all of which are based on the assumption that 80% of humans use LLINs ($C_h = 0.8$). Predictions for toxic-deterrent hybrid product profiles ($\theta_{\mu,pre} = 0.5, \theta_{\mu,post} = 0, \theta_A > 0$) converge with those for purely deterrent product profiles ($\theta_{\mu,pre} = \theta_{\mu,post} = 0, \theta_A > 0$) once deterrence reaches 100% efficacy and prevents any fatal contact with the active ingredient ($\theta_A = 1$ so that $\gamma_{h,p} = 0$). This is to say that given maximum diversion, the probability that a mosquito would attack a covered host becomes zero. A number of further observations suggest this trade-off should be carefully considered when defining a target product profile for developing or selecting a malaria vector control pesticide formulation.

A partially efficacious but purely fast-acting toxic product ($\theta_{\mu,pre} = 0.5, \theta_{\mu,post} = 0, \theta_A = 0$) consistently delivers better protection of non-users than a completely efficacious but purely deterrent ($\theta_{\mu,pre} = 0, \theta_{\mu,post} = 0, \theta_A = 1.0$) product (Figure 3). A reasonable degree of community-level protection for non-users is accrued where attractive, non-human hosts exist for diverted mosquitoes to feed upon. However, in the absence of such alternative blood sources, the unprotected minority of the human population suffers greater exposure and this negative externality increases with increasing deterrence (Figure 3). Furthermore, the consistently strong community-level benefits obtained by non-users when their neighbours use pesticide products with purely toxic activity profiles are undermined in all scenarios by supplementing these lethal effects with increasing levels of deterrence (Figure 3).

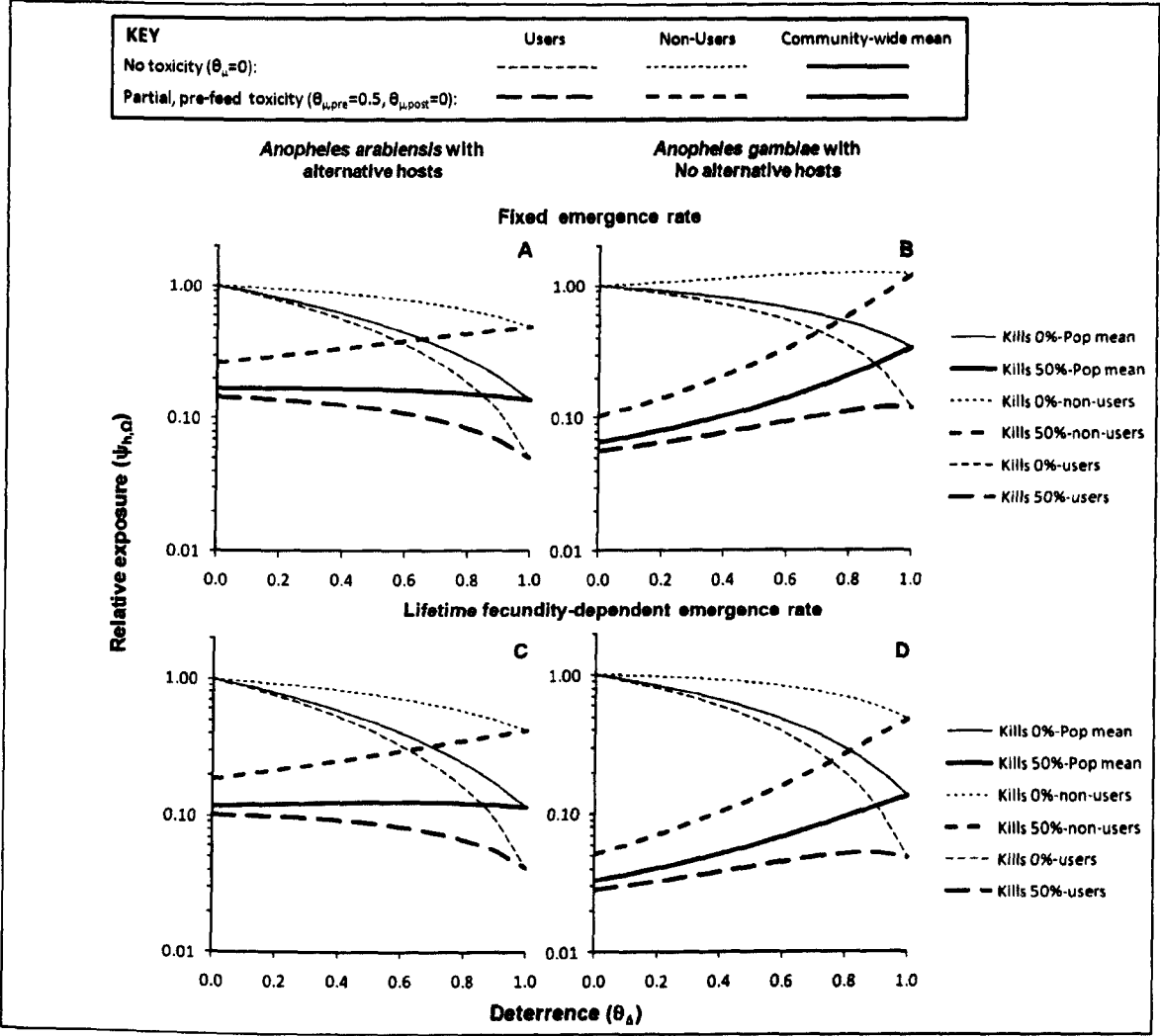


Figure 3. Predicted impact of increasing levels of deterrence (θ_{Δ}) upon exposure to malaria transmission for LLIN or IRS products with ($\theta_{\mu,pre} = 0.5, \theta_{\mu,post} = 0$) and without ($\theta_{\mu,pre} = 0, \theta_{\mu,post} = 0$) toxic properties, assuming either fixed or survival-dependent emergence rates (E) at 80% crude coverage ($C_h = 0.8$).

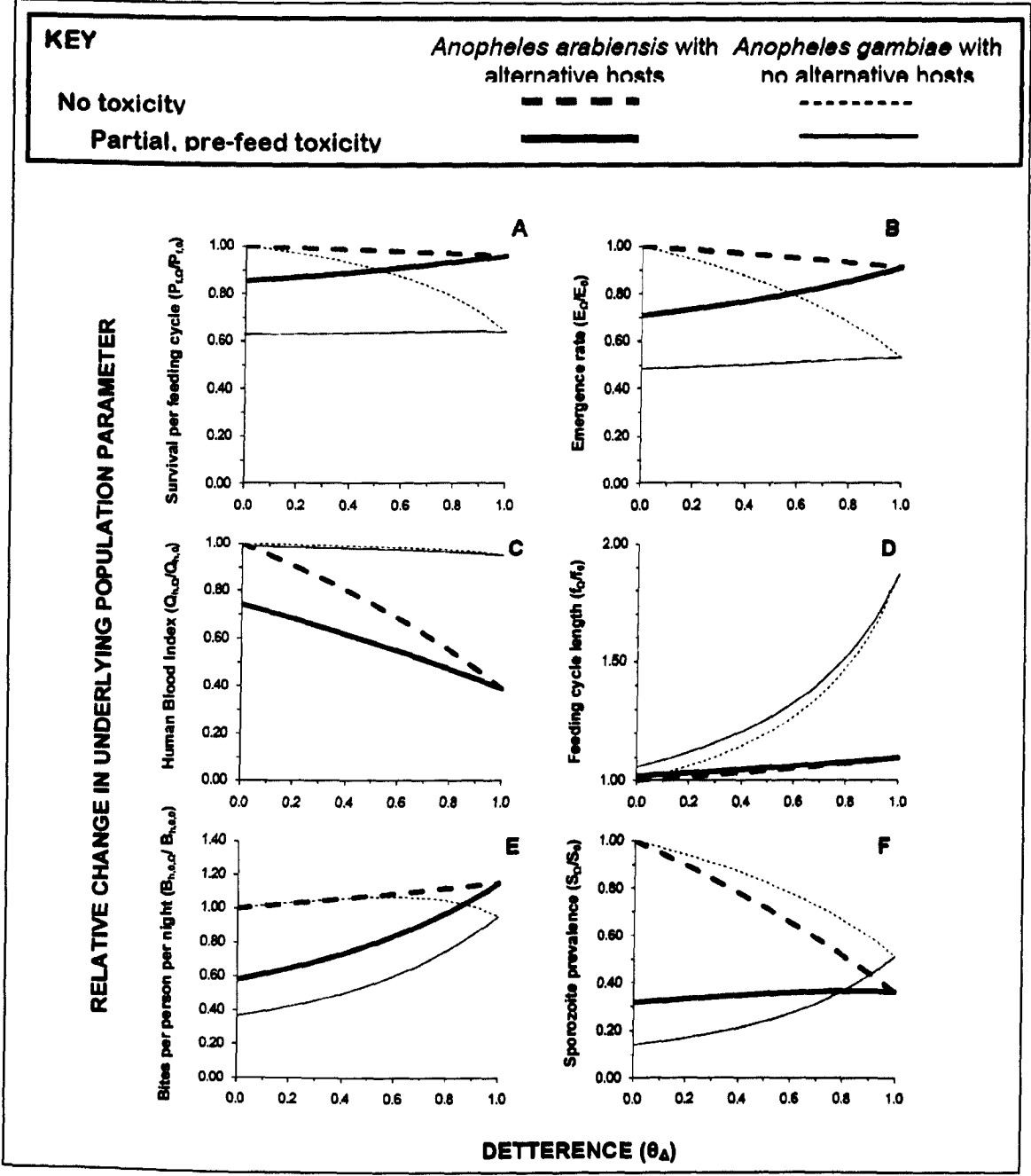


Figure 4. Predicted impact of increasing levels of deterrence (θ_A) upon underlying biodemographic mosquito and sporogonic-stage parasite population parameters that determine malaria transmission for LLIN or IRS products with ($\theta_{\mu,pre} = 0.5, \theta_{\mu,post} = 0$) and without ($\theta_{\mu,pre} = 0, \theta_{\mu,post} = 0$) toxic properties at 80% crude coverage ($C_h = 0.8$). Only the model with survival-dependent emergence rates (E) is presented.

Where alternative animal hosts exist, benefits for users of toxic nets are modestly enhanced as high levels of personal protection provided by strong deterrent properties ($\theta_d > 0.5$) are realized (Figure 3). However, this results in an approximate break-even scenario, in terms of mean relative exposure across the entire community because increased benefit for users is offset by reduced benefit for non-users (Figure 3). Where alternative sources of blood are absent, increasing deterrence actually progressively undermines protection of users because the increased personal protection conferred is more than counterbalanced by dramatically attenuated community-level impact (Figure 3).

Note that for all of these conclusions, the model which includes survival-dependent emergence rates (Figure 3 c and d versus a and b) improves the predicted outcomes for purely deterrent products and toxic-deterrent hybrids but in no case does so dramatically enough to alter the overall trend or conclusions reached (Figure 3). These simulations suggest that purely toxic products are preferable to purely deterrent ones and that enhancing the personal protection afforded by a toxic product by increasing its repellent or irritant properties will consistently undermine or even reverse communal protection of non-users. In fact, where vectors lack alternative non-human hosts, increasing deterrence may even undermine benefits for users because the degree of community-level protection obtained with purely toxic products is far greater than personal protection at the high levels of coverage now considered as healthy targets for any malaria control programme [1, 2].

Figure 4 illustrates how such counterintuitive predictions may be rationalized by examining the underlying biodemographic parameters describing the vector and sporogonic-stage parasite populations, which ultimately determine impact on malaria transmission. Vector survival per feeding cycle (P_f) is the most important single determinant of malaria transmission intensity other than temperature and is substantially reduced by toxic, deterrent

and toxic-deterrent hybrid products where no alternative blood hosts exist (Figure 4a). Where alternative hosts occur, only toxic products with little or no deterrence are predicted to usefully reduce vector survival (P_f). Regardless of whether alternative hosts are present, increasing deterrence of toxic products consistently weakens impact upon this most important target for adult malaria vector control, modest reductions of which result in quasi-exponential suppression of transmission [4, 15, 39]. As the impact upon vector reproduction (E) has been modelled as a linear function of the number of bites taken per lifetime (b_h), itself a simple function of survival (P_f) [15], it is unsurprising that the impact of these various product profiles mirrors that upon survival (Figure 4b). Being a squared term in all malaria transmission models [4, 30, 39], the proportion of blood meals that the vector population obtains from humans is the next most important determinant of malaria transmission intensity at global [43] and local level [15, 39, 42]. Where alternative sources of blood are available, deterrence can dramatically reduce this target parameter in its own right and also enhances the impact of toxic products when added as a supplementary activity (Figure 4c). In the absence of alternative hosts, no toxic, deterrent or hybrid product has any meaningful impact on this target parameter. Consistent with outputs of previous formulations [15], increasing deterrence can greatly extend the feeding cycle length (f) of the vector where no alternative non-human hosts exist but has a very modest effect where they are present (Figure 4d). Consistent with the recently revised, distinct definitions of host and blood availabilities [24], toxicity has no influence on this determinant of mosquito survival (P_f), feeding frequency ($1/f$), reproduction (E) and transmission potential (b_h , S).

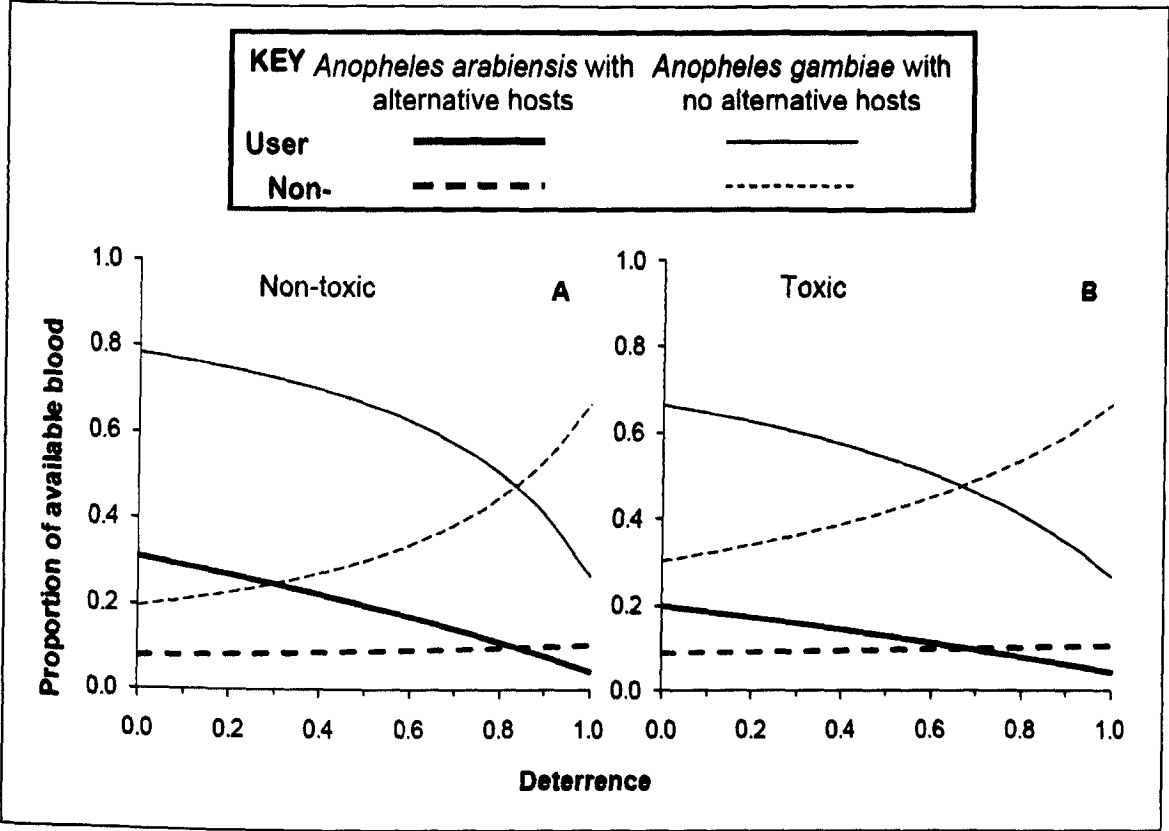


Figure 5. Predicted impact of increasing levels of deterrence upon the share of total blood availability (Z) that human users and non-users of LLINs (Z_h) constitute as the deterrence of an LLIN or IRS product at 80% crude coverage ($C_h = 0.8$).

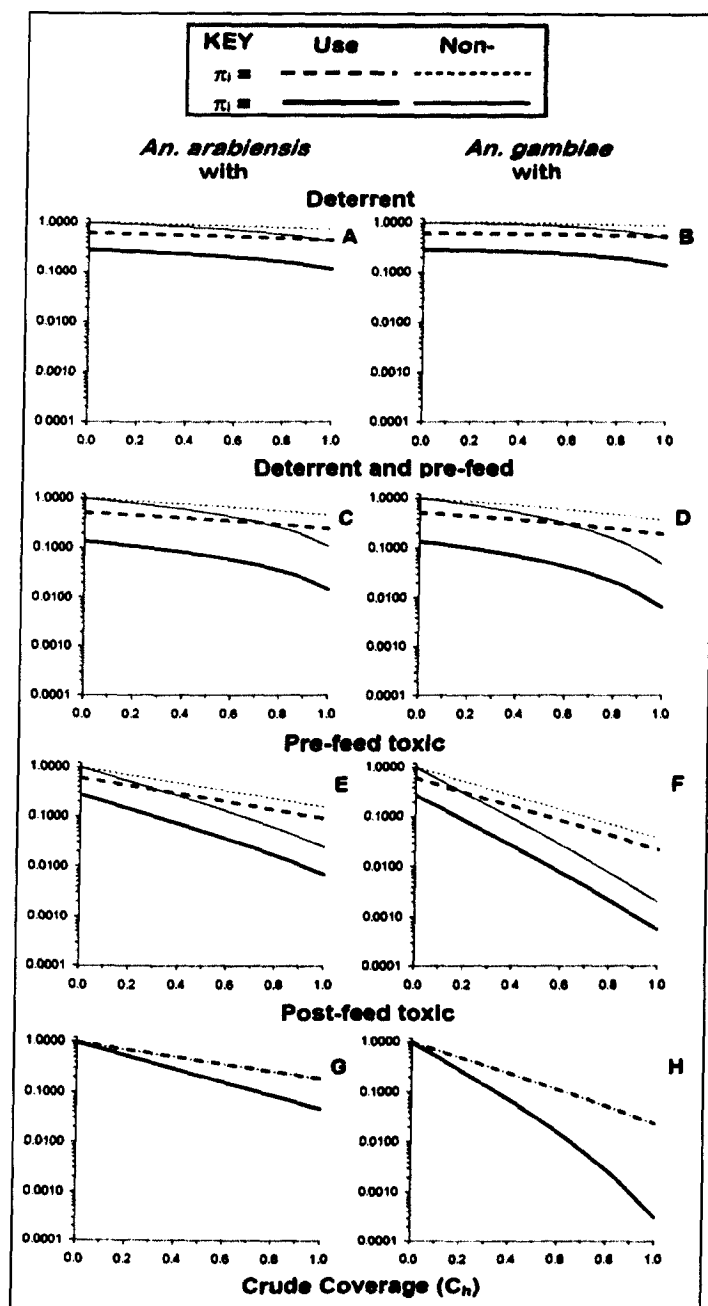


Figure 6. Predicted impact of LLIN or IRS products with purely pre-feeding toxic ($\theta_{\mu,pre} = 0.8, \theta_{\mu,post} = 0, \theta_{\Delta} = 0$), post-feeding toxic ($\theta_{\mu,pre} = 0, \theta_{\mu,post} = 0.8, \theta_{\Delta} = 0$), deterrent ($\theta_{\mu,pre} = 0, \theta_{\mu,post} = 0, \theta_{\Delta} = 0.8$) and pre-feeding toxic-deterrent hybrid ($\theta_{\mu,pre} = 0.8, \theta_{\mu,post} = 0, \theta_{\Delta} = 0.8$) properties upon malaria transmission exposure for users and non-users where either most ($\pi_t = 0.9$) or half ($\pi_t = 0.5$) of baseline transmission occurs indoors.

In summary, toxic products consistently reduce vector survival and reproduction rates, especially in the absence of alternative blood sources. In places where such non-human preferred hosts exist, toxic products only reduce the proportion of blood meals that are human but have no impact on vector feeding cycle length. In contrast, purely deterrent products only have useful impacts upon the proportion of blood meals obtained from humans where alternative hosts exist and upon feeding cycles length where they are absent. Deterrent products, therefore, impact one of these two target parameters or the other and it is notable that neither has as strong an influence upon transmission as survival, particularly when further impact upon mosquito reproduction rates is considered.

By definition (Figure 1), increasing deterrence of a product inevitably increases the proportion of available blood that non-users constitute at any given coverage level (Figure 5) and therefore the share of mosquito bites they experience, regardless of whether that product is toxic or not. When the predicted extent of this inequitable redistribution of biting mosquitoes (Figure 5) is combined with the predicted impacts upon the biodemographic properties of the vector population (Figure 4 a to d), the overall impact is to increase biting rates for non-users (Figure 4e) even where alternative blood sources are absent so vector survival (Figure 4a) and reproduction (Figure 4b) are reduced because the availability of blood becomes limiting. This effect is so dramatic that, even for toxic products, increased exposure of non-users to bites can occur at high levels of deterrence ($\theta_d > 0.8$). While such negative externality in the form of diverting biting mosquitoes to unprotected non-users has been envisaged and discussed previously, the simulated impact of increasing deterrence of toxic products upon the sporozoite

infection prevalence are perhaps more interesting. Consistent with previous simulations [15], purely deterrent products consistently reduce sporozoite prevalence (Figure 4f) by either lowering human blood indices where alternative hosts are available (Figure 4c) or reduce survival (Figure 4a) and extend feeding cycle length (Figure 4d) where they are not. More surprising is the prediction that increasing the deterrence of a toxic product can attenuate impact upon sporozoite prevalence. In the case of vector populations lacking an alternative non-human host, this rebound of sporozoite infection prevalence arising from enhancing the personal protection provided by the product, by increasing irritant or repellent properties, is quite substantial. In fact this weakening of impact upon sporozoite prevalence may be as important a contributor to the dramatic attenuation of overall impact upon transmission (Figure 3b and d) as redistribution of bites to unprotected non-users (Figure 5).

Figure 6 illustrates just how much more efficacious a purely toxic product can be. In both vector-host scenarios, toxic (Figure 6c and d) or toxic-deterrent hybrids (Figure 6e and f) are clearly superior to non-toxic deterrent products (Figure 6a and b). Obviously, the toxic but not deterrent product confers less personal protection than the toxic-deterrent hybrid but correspondingly provides the best communal protection for non-users as coverage increases. Even in the *Anopheles arabiensis* scenario where alternative hosts are available, the benefit to users of a purely toxic product arising from combined personal and community-level protection exceeds that of a toxic-deterrent hybrid at 57% coverage where baseline transmission primarily occurs indoors ($\pi_i = 0.9$) and only 27% coverage where an equal amount of baseline transmission occurs outdoors ($\pi_i = 0.5$). For *An. gambiae*-dominated transmission systems without alternative blood

hosts, the advantage of purely toxic products conferring less protection than those supplemented with deterrence is even more dramatic and obvious, with almost three orders of reduction of transmission possible within feasible coverage targets and the purely toxic product providing greater protection than the hybrid at 22 and 12% coverage, respectively, where most ($\pi_t = 0.9$) and half ($\pi_t = 0.5$) of baseline transmission occurs indoors. Not only do purely toxic products have greater efficacy at reasonable coverage levels, they are also more robust to attenuation by outdoor-feeding behaviours in the target vector population ($\pi_t = 0.5$) because, under such conditions, deterrent products simply divert mosquitoes to feeding on users at times when they are unprotected, especially when no alternative non-human hosts are available.

With the exception of the two bottom panels of Figure 6, all toxic actions simulated thus far are assumed to kill mosquitoes before they can bite the occupant of the house or net. This kind of scenario is best reflected in reality by LLINs with which the pyrethroid insecticide activity is specifically applied to a physical barrier between the attacking mosquito and the protected host so that most dead mosquitoes collected in experimental hut trials are unfed. However, in the case of IRS with non-deterrent insecticides, such as entomopathogenic fungi [44], bendiocarb [19], chlorpyrifos methyl [45], and even pyrethroid-based LLINs that have been depleted of insecticide after several years of use [16], most mosquitoes killed succeed in feeding before dying so little, if any, personal protection is conferred. Figure 6 G and H represent such a scenario and this is reflected in the fact that the predicted degree of protection of users and non-users is identical because this is exclusively mediated by community-level suppression of transmission. Obviously, a purely insecticidal product which kills mosquitoes fast enough

to prevent blood feeding and therefore also confers personal protection (Figure 6E and F) is preferable to one that kills them afterwards and does not (Figure 6G and H).

Nevertheless, even a purely toxic product, which confers no personal protection because it only kills mosquitoes after they have fed (Figure 6G and H), is a consistently better option in terms of protection of non-users than products with deterrent properties, regardless of whether (Figure 6C and D) or not (Figure 6A and B) that product also has insecticidal activity that kills mosquitoes before feeding. Comparing the residual transmission levels achieved with products that confer only community-level protection through purely post-feeding toxicity with that attained by more conventional products with purely deterrent or deterrent plus pre-feeding insecticidal activities (Figure 7), shows that the non-user is always better off with the former. For zoophagic vectors with alternative hosts available that predominantly feed indoors ($\pi_i = 0.9$), deterrent plus pre-feeding insecticidal activity attains lower residual transmission for users than purely post-feeding insecticidal activity. However, such a scenario with most of the alternative hosts and mosquito feeding activity occurring indoors is probably unusual and occurs in a limited number of settings across the tropics.

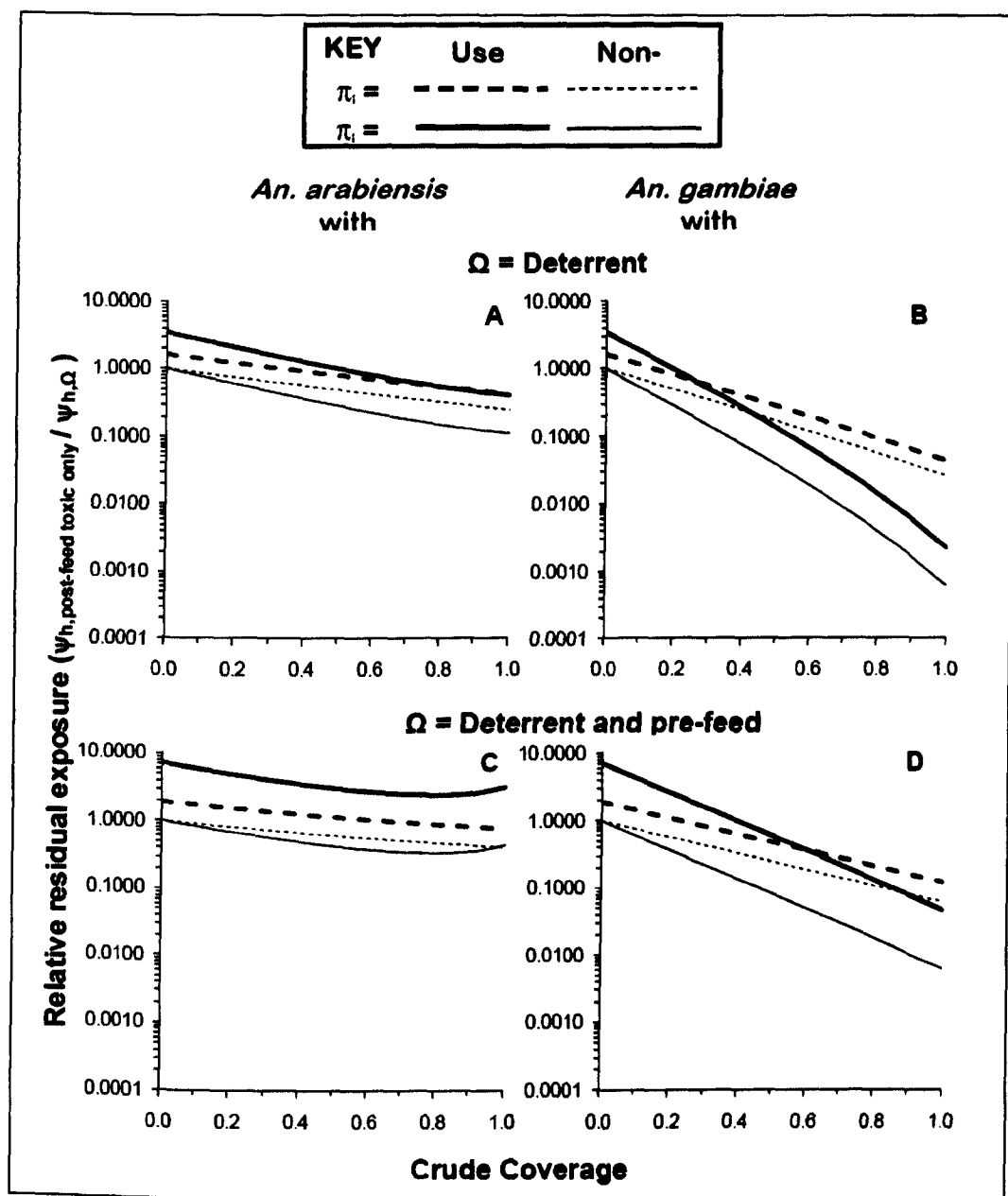


Figure 7. Relative residual malaria transmission achieved with varying levels of crude coverage of a purely post-feeding toxic LLIN or IRS product ($\theta_{\mu, \text{pre}} = 0, \theta_{\mu, \text{post}} = 0.8, \theta_{\Delta} = 0$), compared with products with both purely deterrent ($\theta_{\mu, \text{pre}} = 0, \theta_{\mu, \text{post}} = 0, \theta_{\Delta} = 0.8$) and pre-feeding toxic-deterrent hybrid ($\theta_{\mu, \text{pre}} = 0.8, \theta_{\mu, \text{post}} = 0, \theta_{\Delta} = 0.8$) properties for users and non-users where either most ($\pi_i = 0.9$) or half ($\pi_i = 0.5$) of baseline transmission occurs indoors.

In all other scenarios, especially where half of transmission occurs outdoors ($\pi_i = 0.5$), the purely pre-feeding insecticide confers superior overall protection to users despite complete lack of personal protection once a minimum coverage threshold is surpassed. Compared with pure deterrents, overall protection of users becomes greater for the purely post-feeding insecticidal product at quite modest crude coverage levels (49 and 20% for *An. arabiensis* with alternative hosts and *An. gambiae* without them, respectively) where most transmission occurs indoors ($\pi_i = 0.9$) and even lower thresholds (35 and 14%, respectively) where outdoor feeding and/or resting is more common ($\pi_i = 0.5$). Compared with products combining deterrent with pre-feeding insecticidal activity analogous to LLINs, similar patterns were observed, with the consistent disadvantage of purely post-feeding toxicity where alternative hosts exist and most transmission occurs indoors being reversed when outdoor transmission becomes important and crude coverage exceeds 65%, while it becomes consistently advantageous for vectors lacking alternative non-human hosts at remarkably low coverage thresholds of 39% for predominantly indoor transmission and only 22% where half of transmission occurs outdoors.

Discussion

The idea that deterrence reduces the impact of toxic activities of pesticides upon mosquito survival is long-established [46] and was discussed extensively during the previous global campaign to eradicate malaria [47-49] as well as the beginning of the more recent drive to promote scale up of LLINs and IRS for control purposes [50].

Deliberate design of pesticide-based vector control products to match ideal target product profiles has recently been reprioritized as an important issue [13] now that more ambitious programmes to control, eliminate or even eradicate of malaria are back on the global agenda [51, 52]. The process-explicit model of malaria transmission described here captures the sequential interaction between deterrent and toxic actions of vector control pesticides. In simple terms, it is not realistic to expect that one can discourage mosquitoes from making contact with an active ingredient without compromising the ability of that pesticide to kill them (Figure 1). Sub-lethal exposure that deters mosquitoes inevitably reduces the proportion which is subsequently exposed to higher, lethal doses. In fact, the extreme example outlined on the right hand side of all the panels in Figures 3 and 4, wherein the predicted impacts of products with and without toxic activities converge once 100% deterency is achieved, clearly demonstrates that this is a choice which must be made: increasing deterency and personal protection must always be traded off against reduced toxicity-mediated mosquito mortality and potent communal level protection where high coverage is achieved.

The assumptions and definitions of this model (Figure 1 and Methods) are also fully compatible with recent recommendations that toxic activities and both forms of deterrence, namely contact irritation and spatial repellence, are distinct and that each pesticide-affected mosquito collected in an experimental hut trial should be classified as having either responded in a manner characteristic of only one of these possible outcomes [13]. While parameter estimates from published studies have been deliberately avoided to minimize any appearance of recommending for or against specific commercial product choices, this model can be readily and directly parameterized from existing, standardized

experimental hut evaluations. The diversion term θ_d is estimated directly as the proportional change in the number of mosquitoes which either do not enter the hut (deterrence) or which leave unfed (excito-repellency) but do not subsequently die.

The mortality terms $\theta_{\mu,pre}$ and $\theta_{\mu,post}$ are estimated as the increased proportion of all mosquitoes caught in a hut with a given LLIN or IRS product which were found dead or that subsequently died which were either unfed or fed respectively. However, to enable the application of this model to such experimental hut study outcomes, published summaries will need to explicitly distinguish between pre- and post-feeding mortality [23] and will ideally include the raw data as supplementary online material. The model described also accounts for the distinctive impacts of toxic activities, which kill mosquitoes before or after they have fed upon the occupant of a covered house or sleeping space. A variety of well-established domestic vector control products and emerging new technologies only kill mosquitoes after they have fed because they are applied as IRS formulations or because they are slow acting. Such alternatives to DDT or pyrethroids include entomopathogenic fungi [44], bendiocarb [19], chlorpyrifos methyl [45], and even pyrethroid-based LLINs that have been depleted of insecticide after several years of use [16], can take hours or days to kill most of the exposed mosquitoes but clearly can deliver massive levels of malaria control if sufficient coverage can be achieved. Only two previous models distinguish between the effects of pesticidal products that kill mosquitoes before and after they feed upon humans [14, 53]. While one only considers processes that occur in houses and does not capture the community-level effects of different product profiles upon transmission [14], the other does not account for

outdoor biting and, like previous versions of this model [24], inaccurately treats diversion and mortality as independent, rather than sequentially coupled, events [53].

The specific results presented suggest that if high coverage levels can be achieved that are consistent with current World Health Organization targets [1, 2], purely toxic products with no deterrence are predicted to generally provide superior protection to non-users and even users, especially where vectors feed exclusively on humans and a substantial amount of transmission occurs outdoors. Remarkably, this is even the case if that product confers no personal protection and only kills mosquitoes after they have fed. Products with purely mosquito-toxic profiles may be preferable to those with equivalent toxicity but which confer superior personal protection because of higher deterrence for programmes with universal coverage targets. Purely mosquito-toxic products which confer modest personal protection because they lack deterrence, or which confer none because they only kill mosquitoes after they have fed, will therefore require aggressive “catch up” campaigns to achieve high coverage rapidly and behaviour change communication strategies that emphasize the communal nature of protection.

As with all mathematical predictions, these predictions should only be considered as evidence of plausible hypotheses based on simplifying assumptions and imprecise parameterization. Lessons from learned from historic mistakes, specifically setting malaria prevention policy based on overconfident interpretation of malaria transmission models [3, 4], are as relevant today as they ever were [54].

For example, one notable simplification to keep in mind is that complete gonotrophic concordance, meaning that each egg batch requires one and only one blood meal, has been assumed. In reality, the first blood meal typically requires at least one

additional pre-gravid blood meal to achieve mature phase II development of the ovaries [55-57] and additional blood meals may even be taken during subsequent gonotrophic cycles [58]. While such increased feeding frequency would undoubtedly increase malaria transmission intensity in the absence of interventions such as LLINs or IRS, it would also be expected to increase the frequency of contact with such measures that mosquitoes would be exposed to early in their lives. Correspondingly, incorporating these subtle aspects of mosquito behaviour would most probably enhance the predicted impact of these measures upon transmission and therefore strengthen, rather than weaken, the contrasts between alternative target product profiles suggested here.

The only potentially major inaccuracy that seems obvious from the outputs of this model lies in the prediction that purely deterrent products will provide weak communal protection for non-users and may even increase their exposure. While this phenomenon appears plausible in theory and has been documented by field trials of some topical repellents [21], the experimental design of that study define situations in which only single users were protected, equivalent to negligible community level coverage ($C_h \approx 0$) so community-level effects were neither realized nor evaluated. Furthermore, these predictions seem slightly at odds with observations from field trials of community-wide use of essentially untreated mosquito nets in both Tanzania [59] and Papua New Guinea [60]. In both cases, high coverage of nets lacking meaningful pesticidal properties but deterring mosquitoes through simple physical barrier effects successfully reduced malaria transmission.

Combined with the anecdotal but reasonable attribution of reduced malaria transmission in many settings to housing improvements conferring similarly direct

protection through similar physical barriers [61], these net trials suggest that the disappointing predictions for purely deterrent products described here should be interpreted with a degree of caution. The most obvious possible explanation for such possible discrepancies is the uncertainty associated with survival rate of mosquitoes foraging for blood or aquatic habitat and the extreme sensitivity of predictions to this parameter value and to baseline total availabilities of these resources [15, 28, 39, 62-64]. To go beyond speculation based on sensitivity analysis of these critical but, as yet, unmeasured parameters, will clearly require the development of robust field methods, notably trapping of gravid *Anopheles* seeking oviposition sites [28].

With some notable exceptions, these simulations compare well with recent, less generalized, modelling analyses which examine choices between specific product types and combinations thereof [65, 66]. Deliberately, no specific product has been named, nor has any measured parameter value for any specific product been set in any of these simulations. Instead, the product parameters have been tuned them across the full range of possible values so that ideal target product profiles can be objectively outlined for manufacturers and their clients to aim for prospectively rather than restrict discussion to the relative merits of currently available products and product combinations. Nevertheless, the parameter space explored here encompasses all the specific examples of product types evaluated in recent modelling analyses [65, 66], resulting in predictions which are readily comparable in broad terms (Figures 3 and 6). Both these complementary recent studies [65, 66] also conclude that IRS with a highly deterrent product such as DDT will have less impact than a predominantly insecticidal product such as IRS with bendiocarb or pyrethroid-based LLINs. However, their conclusions

regarding combining such product types differ somewhat and the existing evidence base is insufficient to inform which of these three formulations appears most accurate. Chitnis *et al.* predict that supplementing a predominantly insecticidal LLIN products [65] with a highly deterrent one such as IRS with DDT will have a larger impact upon transmission than either one as a stand-alone measure. In contrast, the simulations of Yakob *et al.* [66], suggests the opposite: that placing a deterrent product in the same house as a predominantly insecticidal one will undermine the superior impact of the latter for the same reasons outlined here and captured in convergence of outcomes with toxic and non-toxic products in Figure 3.

Perhaps the most important observation about the lack of consensus between these three model formulations is that sufficient field data do not exist to reliably compare them in terms of their predictive value. Recent reviews of the impact of IRS [10], and specifically IRS combined with insecticidal nets [10, 67], both conclude that rigorous, large-scale, randomized controlled trials are conspicuous by their absence. An abundance of descriptive studies unambiguously demonstrate that IRS has massive overall impact and that combining with ITNs gives generally improved personal protection [10, 49]. To the knowledge of the authors, however, no study yet exists in which the exclusively communal protection afforded to residents of unsprayed houses in IRS programmes has been measured as rigorously as it has for non-users of insecticidal nets in communities with high coverage levels [11, 60, 68-72]. Given these limitations in the evidence base for IRS as a stand-alone prevention strategy, it is perhaps unsurprising that the evidence base to support decisions about combining LLINs and IRS is

insufficient and has become a common point of discussion for both theoreticians and practitioners [10, 65-67].

Despite these limitations in both the consistency of outputs from alternative existing models and the empirical evidence base from the field, important lessons can be learned from these simulations which are intuitive and for which no caveats seem obvious. Although no evidence, based on rigorously randomized trials, for the *probability* of the deterrence-related attenuation of insecticidal impact have been reported, the existing descriptive evidence base presents a strong case for the *plausibility* [73] of this phenomenon.

The effect of insecticidal attack was enhanced by use of non-irritant insecticides [49]

In fact, the ideal target product profile outlined here was already suggested during the previous malaria eradication era, when the impact of DDT which has a mixed deterrent-plus-toxic profile, was contrasted with that of Dieldrin which acts by contact toxicity only [13]:

*In many instances, Dieldrin proved to be more effective than DDT, but its higher cost, its toxicity to mammals, and the fast-spreading resistance of *Anopheles gambiae* to this insecticide limited its further use in Africa [49]*

This model presented herein simply strengthens, explains and generalizes the plausibility of this argument, highlighting the lack of affordable, safe alternatives to Dieldrin with similarly non-deterrent properties. Three decades later, with insecticide resistance on the rise [74] and increasing levels of exophagy being reported for residual vector populations in Africa [26] and Asia [27], it is likely that several such active ingredients with distinct, complementary mechanisms will be required to prevent and

manage insecticide resistance in the long term. These simulation results, therefore, serve as a timely reminder of the need for increased investment in development and evaluation of insecticidal products with purely toxic modes of action to achieve improved and sustained malaria vector control.

Even if the worst-case scenarios predicted here are confirmed through large-scale trials, it is important to remember that this analysis is restricted to typical LLIN or IRS products that are used indoors. One of the most interesting phenomena that this model captures, which is increasingly relevant as the importance of outdoor-biting vectors is recognized [26, 27, 54, 75], is that the advantage of purely toxic products becomes greater where vector mosquitoes tend to feed outdoors (Figures 6 and 7). This suggests that deterrent activities can not only divert mosquitoes to animals or to humans lacking such products but also to the users themselves at times of the day when they are outside of the house and unprotected. This new insight arises directly and intuitively from the reformulation of how coverage and protection have been conceptualized and expressed mathematically. Further extensions of this approach may be useful for examining a wider diversity of possible pesticidal vector control products that target mosquitoes outside of houses [76-78] and even away from humans [24, 79-81]. This conceptual and mathematical formulation represents a useful new tool for rational design of malaria vector control products. Furthermore, the way in which coverage and protection are conceptualized here represents a substantive change in thinking that may also enable more lucid re-examination of what these terms really mean in practice [82, 83].

Competing Interests

None

Acknowledgements

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Authors' Contributions

All authors formulated the research question and developed the conceptual basis of the model. GFK drafted the model formulation and manuscript in consultation with FOO, SJM and NC. The contents are the responsibility of GFK, NC, SJM and FOO and do not necessarily reflect the views of USAID, the United States Government or the Bill &

Melinda Gates Foundation. All authors have read and approved the final version of the manuscript.

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Chapter VIII

Simulated community-level effects of combining long lasting insecticidal nets with indoor residual spraying for malaria control in Africa^{*}

Abstract

Background: Even though it is common practice to combine indoor residual spraying (IRS) with long lasting insecticide nets (LLINs) in highly endemic communities, there is limited evidence to suggest that such strategies confer greater protection against malaria than either method when used alone. Experimental hut trials have already demonstrated improved personal and household level protection with certain LLIN/IRS combinations, but it remains unclear whether such findings can also translate to proportionately greater benefits at community level.

Methods: an existing deterministic mathematical model of mosquito life cycle processes is adapted and used to estimate how malaria transmission might be affected, if LLINs are combined with IRS, and whether such combinations would be synergistic or redundant, relative to the use of either method alone. The model was modified to allow use of data derived directly from experimental hut evaluations where untreated bed nets are used as the experimental controls. A scenario was simulated to represent a closed community where residents own cattle, and where the main malaria vector is *Anopheles arabiensis*, an increasingly dominant vector species in Africa, which remains a significant challenge to

^{*} Adapted from: Okumu FO, Moore SJ, Killeen GF: **Simulated community-level effects of combining long lasting insecticidal nets with indoor residual spraying for malaria control in Africa.** *Manuscript in Preparation*

control even with high LLINs and IRS use rate. Considering situations with either LLINs or IRS as the pre-existing intervention, we then calculated a relative improvement in transmission control achievable when the complementary intervention is introduced.

Findings: Transmission control is improved when the common pyrethroid based LLINs are added onto IRS treatments such as actellic and lambda cyhalothrin, but not DDT, which is known to be less toxic but highly deterrent against mosquitoes. On the other hand, the outcome remains unchanged when lambda cyhalothrin IRS is added to communities already using LLINs. Nevertheless, addition of highly toxic IRS such as with actellic vastly improves transmission control relative to just the LLINs alone.

Conclusions: This *in-silico* assessment shows that whereas introduction of LLINs into communities with pre-existing IRS will generally result in improved control of malaria transmission, introduction of IRS into communities with pre-existing LLIN use will most likely be redundant unless the IRS is highly toxic to malaria mosquitoes.

Background

One of the main challenges facing malaria vector control today is the inadequacy of empirical evidence to ascertain potential synergies or redundancies in combining long lasting insecticidal nets (LLINs) with indoor residual insecticide spraying (IRS) [1]. In addition to a few field trials that are now being conducted in malaria endemic countries, mathematical models are becoming increasingly useful for purposes of simulating the effects of such combined interventions [2-4].

In this article, we use an optimised version of a deterministic model based on mosquito life cycle processes, to estimate how malaria transmission might be affected, if LLINs are combined with IRS. For this purpose, we consider closed communities dominated by *Anopheles arabiensis* as the main malaria vector. The model version used here is a hierarchical improvement of versions that have previously been used for a number of purposes including *inter alia*: 1) to compare impacts of LLINs when targeted to all age-groups as opposed to coverage of only pregnant women and children [5], 2) to estimate effects of combining LLINs with odour-baited mosquito traps [6], 3) to assess the extents of exposure to malaria that occurs outside human houses [7], and 4) to assess tradeoffs between repellent and toxic properties of vector control insecticides [8].

To achieve the current objective, additional adaptations of the model were introduced to allow the use of entomological measurements obtained directly from experimental hut studies such as those described earlier (Chapter IV), regardless of whether the huts had been used as controls (e.g. huts fitted with untreated nets only) or as treatments (e.g. huts fitted with candidate LLINs or IRS applications). This way, the optimised version of the model allows incorporation of baseline physical protection

(direct protection from bites) offered by untreated bed nets (where these are used as the experimental controls), in addition to the protection that occurs as a result of the insecticidal active ingredient itself.

Methods

Model description

A detailed description this hierarchical model, which has been incrementally improved over time and details of its previous applications, can be obtained from previous publications [6, 8, 9]. In this section, we describe specific adaptations of the model for purposes of estimating incremental community level effects of combining LLINs with IRS. Modifications to the original formulation [8, 9] were introduced to enable direct input of data from standard experimental hut evaluations of intradomiciliary vector control methods [10, 11]. Unlike all previous versions, these latest modifications recognize the fact that untreated mosquito nets, commonly used as ‘experimental controls’ in hut studies actually also provide a certain level of basic protection, mainly because they physically obstruct mosquitoes attempting to obtain blood meals from persons sleeping under the nets.

To represent the total protection attainable from IRS or nets, the process leading to attack and feeding by host seeking mosquitoes upon hosts was redefined such that for vector control interventions that can divert mosquitoes from actually reaching a human hosts inside a house, the diversion process was subdivided into two phases (Figure 1). The first is the diversion that occurs outside the house as mosquito attempts to enter a house with the intervention (Δ_{outdoors}). The second is the diversion that occurs indoors

when the mosquito has already entered the house to attack a human inside (Δ_{indoors}). Therefore, using the example of a bed net as a personal protection measure, we can say that for a mosquito to successfully attack any human using the bednet, i.e. an attack upon a protected net user, that mosquito must not have been diverted outdoors prior to entering the house, and it must also not have been diverted indoors prior to biting the net user. Unlike in previous model versions, wherein this second level of diversion was not explicitly identified, the attack probability is hereby redefined as the remaining fraction of mosquitoes encountering the host-occupied house that are unaffected by these two sequential diversionary processes:

$$\gamma = (1 - \Delta_{\text{outdoors}})(1 - \Delta_{\text{indoors}}) \quad (\text{Eq. 1})$$

The above equation assumes that most female malaria vectors entering human occupied houses do so with a sole intention of attacking and obtaining blood meals from the human host inside. Nevertheless, we can directly input counts of mosquitoes caught inside or exiting experimental huts, regardless of whether those huts were fitted with only untreated mosquito nets (control huts) or LLINs and IRS (treatment huts). Moreover, the revision allows us to unambiguously distinguish long-range spatial repellence, where mosquitoes are diverted at a distance before they enter huts, and also short range deterrence and contact irritant effects, where the interventions force mosquitoes that come into huts to exit those huts without feeding [1]. Due to ethical constraints upon using a truly representative negative control with no protection whatsoever [12], all our field experimentations, from which we draw the data for the simulations reported here, were conducted using intact untreated nets as controls, instead of absolute ‘zero-protection’ controls. For purposes of this model, it is therefore assumed that in the houses

where the only intervention used is the untreated net, i.e. where there is no chemical-induced long-range repellence, no diversion occurs outdoors ($\Delta_{\text{outdoors}} = 0$) and therefore the number of mosquitoes caught inside those huts would be approximately similar to the number of mosquitoes caught in houses with no intervention at all.

The diversion that occurs prior to house entry is therefore calculated as:

$$\Delta_{\text{outdoors}} = 1 - \frac{M_p}{M_0} \approx 1 - \frac{M_p}{M_1} \quad (\text{Eq. 2})$$

where M_p refers to total catch of a given malaria vector species in a given experimental hut or set of huts, M_0 refers to the total catch of the same vector species that would be obtained in the same experimental hut or huts if no protective intervention was used (true negative control) and M_1 refers to the total catch of the same vector species that would be obtained in the same experimental hut or huts if only an untreated bed net was used (pseudo-negative control). The subscript p refers to the different types of interventions, which can be used in houses, and which can take coded values 0, 1, 2.. to n , where 1 represents untreated bed nets, being considered in this case as the most basic form protection against mosquito bites, and is assumed to have negligible impact upon house entry by mosquitoes (i.e. $M_0 \approx M_1$). On the other hand, the diversion that occurs indoors is calculated to represent number of malaria vector mosquitoes that actually enter the huts but which do not attack the host:

$$\Delta_{\text{indoors}} = 1 - \frac{M_{p,\gamma}}{M_p} \quad (\text{Eq. 3})$$

where $M_{p,\gamma}$ refers to the total number of malaria vectors that are considered to have entered the huts and attacked the human inside that hut. In practice, if we were to collect a given number of mosquitoes from a human occupied experimental hut having

any intervention, say IRS, LLIN, both IRS and LLINs or simply untreated nets, it would be possible to classify all the collected mosquitoes (M_p) as either: a) unfed and alive ($M_{uf,l}$), meaning that they did not attack the host inside the hut and are therefore assumed to have been deterred from attacking, b) unfed and dead ($M_{uf,d}$), meaning they attacked the host and died in the process without obtaining a blood meal, c) fed and alive ($M_{f,l}$), meaning that they attacked the host but survived and successfully obtained blood meal, or d) fed and dead ($M_{f,d}$), also meaning that they attacked the host, successfully obtained blood meal but then died, presumably as a result of the attack (Figure 1).

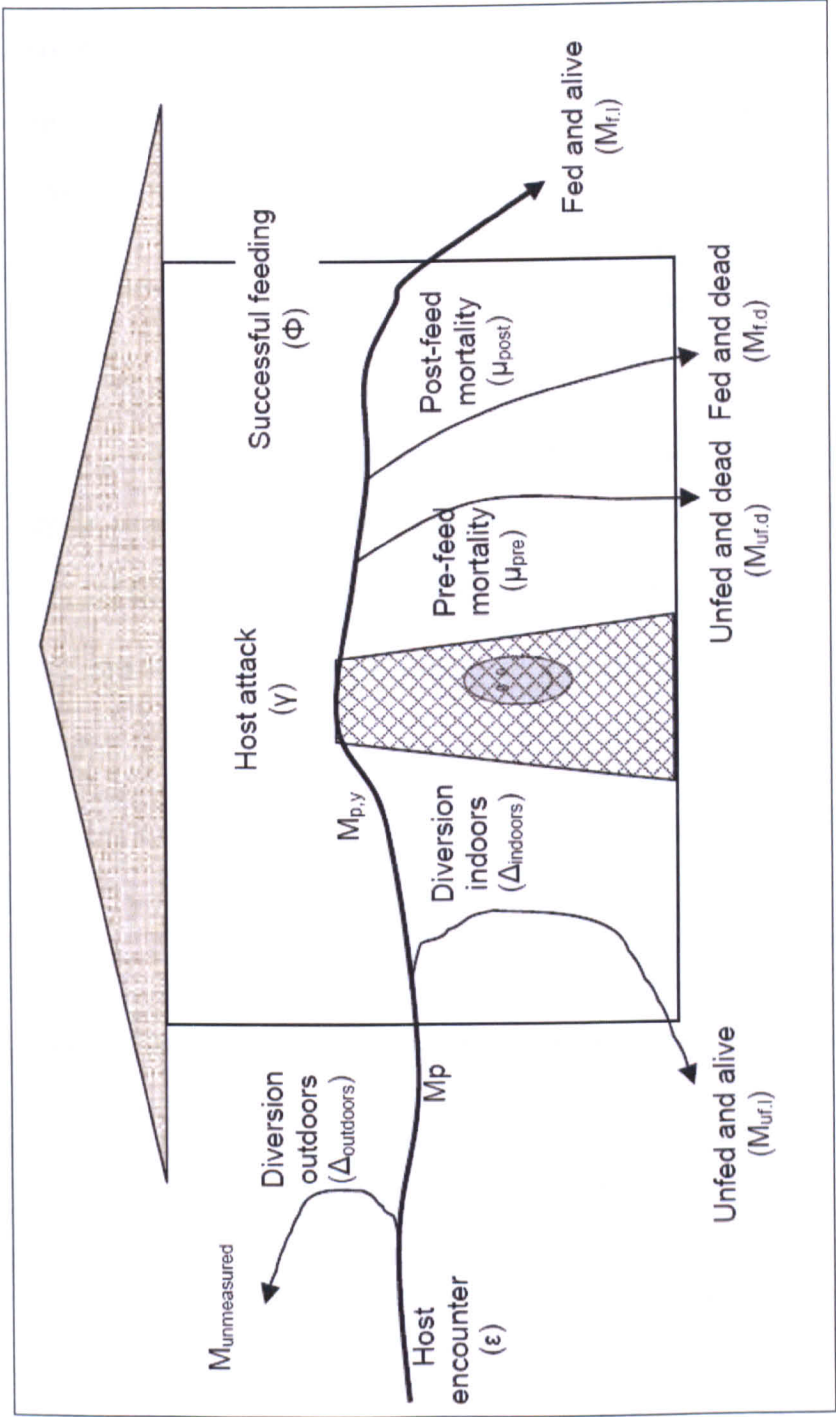


Figure 1 Diagrammatic representation of mosquito host seeking processes as quantifiable in standard experimental hut studies. All mosquitoes caught in the huts and in exit traps attached to the hut are considered as having entered the hut and therefore not diverted outdoors. M_p refers to Mosquitoes entering the huts while $M_{p,y}$ refer to Mosquitoes attacking the host inside the hut.

The parameter, $M_{p,\gamma}$ in equation 3, therefore includes both fatal attacks, represented by dead mosquitoes that are either unfed ($M_{uf,d}$), or fed ($M_{f,d}$), and non-fatal attacks, which are represented by live mosquitoes that that are fed ($M_{f,l}$). It however excludes the unfed mosquitoes that remained alive ($M_{uf,l}$), which in this case are considered the ones which did not attack the host. Equation 3 can therefore be broken down as follows:

$$\Delta_{indoors} = 1 - \frac{M_{p,\gamma}}{M_p} = 1 - \frac{M_{uf,d} + M_{f,d} + M_{f,l}}{M_p} = \frac{M_{uf,l}}{M_p} \quad (\text{Eq. 4})$$

Similarly, we have previously explained that in a single mosquito feeding cycle, attack related mortality can occur either before (μ_{pre}) or after successful feeding (μ_{post}) [8]. In practice both μ_{pre} , and μ_{post} can be calculated directly from experimental hut data as fractions of the number of mosquitoes that attacked the host inside the huts.

$$\mu_{pre} = \frac{M_{uf,d}}{M_{p,\gamma}} \quad (\text{Eq. 5})$$

$$\mu_{post} = \frac{M_{f,d}}{M_{p,\gamma}} \quad (\text{Eq. 6})$$

The combined probability of attack related mortality is calculated as the proportion of all attacks that are fatal.

$$\mu = \mu_{pre} + \mu_{post} = \frac{M_{uf,d} + M_{f,d}}{M_{p,\gamma}} = \frac{M_{p,dead}}{M_{p,\gamma}} \quad (\text{Eq. 7})$$

where $M_{p,dead}$, refers to the total number of dead malaria mosquitoes caught inside the hut.

In earlier versions of this model, these mortality probabilities (μ_{pre} and μ_{post}) were combined and treated as a single event, assumed to occur prior to feeding [5, 6, 13]. This approach remains epidemiologically relevant for most contemporary

interventions, given that the post feeding mortality (μ_{post}), which in practice is often measured as mortality within 24 hours, usually occurs within such a short time that those mosquitoes would not have possibly completed the gestation period, returned to a host seeking state or gone ahead to transmit disease to the next host anyway [14]. Moreover, the subdivision of attack-associated mortality into these two components is not necessary for estimating purely community-level protection against transmission which, unlike personal protection is simply a function of overall mortality probability (μ) [9]. This is to say, that while insecticide-related mosquito mortality occurring after the mosquito has fed on the protected host does not contribute to personal protection, it does contribute to community-level suppression of malaria transmission by reducing population mean mosquito survival.

Therefore to fulfil the current objectives, previous interpretations of the terms [6] are retained so that the probability of mosquitoes feeding upon an encountered host (ϕ_p) using a given protection measure (p) is expressed on the basis of both attack probability and the overall mortality probability.

$$\phi_p = \gamma_p(1 - \mu_p) \tag{Eq. (8)}$$

$$\phi_0 = \gamma_0(1 - \mu_0) \tag{Eq. (9)}$$

where subscript, *p*, with values 0, 1....to 'n', (where 0 refers no protection at all, and 1 refers to untreated nets as the only protection) are used to denote subpopulations that are either protected or unprotected

The amendments above effectively render equations 10, 11 and 13 in the previous version of the model [8], unnecessary as the values needed to simulate effects of the interventions are no longer represented by additional probabilities of diversion (θ_Δ) and death before feeding ($\theta_{\mu,pre}$) caused by the deterrent and insecticidal properties of the nets respectively. Instead, the diversion (Δ) and mortality (μ)

parameters are calculated directly from the experimental hut observations as described above.

In Killeen *et al* 2011 [8] and also the other previous versions [6, 7], populations were classified as either having insecticidal nets or not. The term unprotected hosts was used to refer to people not using insecticidal nets, which in practice also included persons sleeping inside experimental huts supplied with non-insecticidal nets. In those earlier versions, we fixed the baseline diversion (Δ_{hu}) and baseline mortalities (μ_{hu}) associated with persons not using insecticidal nets at a specific value [1, 6], without any regard for the fact that even untreated nets, though commonly used as controls in experimental hut studies, can actually elicit a protection [15, 16] greater than the baseline protection that results purely from individual defences of a person not using any protection at all [17]. Here, it is important to emphasise that unprotected human refers specifically to a person inside a house that has not been sprayed with any IRS insecticide and without any net, whether treated or untreated, but that people using untreated nets are actually considered protected. Other than the highlighted changes, the rest of the equations remain exactly as described in the most recent version of this model [8].

Input parameter values

All the basic ecological parameter values used in this model version are similar to the most recent previous application [8]. However to represent the simulated interventions, the following specific changes were made on parameter values: First it was assumed that the total number of mosquitoes entering a house with no intervention at all (M_0) is approximately equal to the total number entering huts with only untreated bed nets (M_1). Thus we used the M_1 values obtained directly from our

experimental hut studies (Chapter IV). Similarly, other M_p values were obtained for the different interventions that we had tested in our experimental hut assays (Chapter IV). This model is designed in such away as to input directly the actual data as obtained from the different experimental huts. Malaria mosquitoes obtained from the different experimental hut were classified as described above and total numbers of mosquitoes found to be unfed and dead ($M_{uf,d}$), or fed and dead ($M_{f,d}$), fed and alive ($M_{f,l}$), or unfed and alive ($M_{uf,l}$), were directly input into the model equations. Since in most cases the feeding rates were so low that the estimated measures of central tendency would always be zero or near zero, we opted to use the actual numbers of mosquitoes as recorded directly from the experimental hut study. The baseline diversion ($\Delta_{h,u}$) and baseline mortality ($\mu_{h,u}$) values for unprotected humans were similarly assumed to be 0.1 as in previous model applications [6, 8] based on historical reports from true negative controls. As a representative epidemiological scenario, we simulated a closed community where residents own cattle, and the malaria vector is *An. arabiensis*, an increasingly dominant vector species in Africa whose behavioural characteristics remain a significant challenge to control using LLINs and IRS when used singly [4, 16, 18]. We set intervention coverage, as calculated based on proportion of people using the intervention, to 80%, consistent with globally agreed targets [19-21].

All the main parameters and their respective values as used in the simulations are described in Table 1 below.

Table 1: Main parameters and parameter values used in the simulations. All the basic ecological parameter values used in this model version are similar to the most recent previous application [8]. Only those parameters that have been introduced or modified in this version of the model are included

| | Description | Source of values |
|----------------------------|--|--|
| Δ_{outdoors} | The diversion that occurs outside the house when the mosquito is attempting house entry | Derived in Eq. 2 |
| Δ_{indoors} | The diversion that occurs indoors when the mosquito has already entered the house to attack the human indoors | Derived in Eq. 3 |
| M | The total number of malaria vectors caught in a given human occupied hut | Implied in Eqs. 2-7 |
| M_0 | The total number of malaria vectors caught in a given hut or set of huts having no protective treatment inside. | Actual numbers obtained directly from unsprayed experimental huts |
| M_l | The total number of malaria vectors caught in a given hut or set of huts having untreated mosquito nets as the only form of protection inside. Subscripts 2..n can be used to denote any other protective measures apart from untreated nets | fitted with untreated nets as in Chapter IV. See additional file S.7.1 M_0 values are considered equivalent to M_l |
| M_p | The total number of malaria vectors caught in a given hut or set of huts having a protective treatment inside | Only data from the second round of spraying was used. Actual number of mosquitoes obtained directly from mosquito catches in experimental huts |
| $M_{p,y}$ | Total number of malaria vectors that are considered to have entered the huts and attacked the humans inside the huts | fitted with the respective LLINs and IRS chemicals, as described in Chapter IV. See additional file S.7.1 |
| $M_{uf,l}$ | Total number of malaria vectors that were caught unfed and remained alived after 24 hours. Classifiable as non-attacking vectors | |
| $M_{uf,d}$ | Total number of malaria vectors that were caught unfed but died within 24 hours. Classifiable as fatal attacks | |
| $M_{f,l}$ | Total number of malaria vectors that were caught when already fed and remained alived after 24 hours. Classifiable as successful attacks | Only data from the second round of spraying was used. |
| $M_{f,d}$ | Total number of malaria vectors that were caught when already fed but died within 24 hours. Classifiable as fatal attacks | |

Simulated interventions

We considered data from our previous experimental hut study, as described in Chapter IV and V, where we had evaluated four net types (three LLINs and a non-insecticidal net) and three IRS insecticides of different classes (one organochloride, one synthetic pyrethroid, and one organophosphate). The LLINs included Olyset® nets (manufactured by A-Z, Tanzania), PermaNet 2.0® nets (Vastergaard, Switzerland) and Icon Life® nets (Bestnet Europe ltd, Denmark). Olyset® nets are made of polyethylene netting (150 denier), impregnated during manufacture with synthetic permethrin at 2% w/w (equivalent to 1000mg of active ingredient/m²). PermaNet 2.0® is a 100%-polyester net (100 denier), coated with 55-62mg of synthetic deltamethrin/m², resulting in insecticide concentrations of approximately 0.14% w/w. Icon Life® is also a polyethylene net and is impregnated during manufacture with synthetic deltamethrin at 0.2% w/w (\approx 65mg of active ingredient/m²). The IRS treatments included DDT wettable powder (AVIMA, South Africa) sprayed at a WHO recommended dose of 2g/m², lambda-cyhalothrin capsule suspension, (Syngenta, Switzerland), sprayed at a dose of 0.03g/m², and pirimiphos-methyl emulsified concentrate, also known as actellic (Syngenta, Switzerland), sprayed at a recommended dose of 2g/m².

These IRS compounds and all the LLINs, except Icon Life®, have been approved by WHO for malaria vector control [22]. DDT (an organochloride) and lambda cyhalothrin (a synthetic pyrethroid) are both commonly used for IRS in Africa, and together with pirimiphos-methyl (a WHO approved organophosphate), they represent a diversity of common insecticide classes currently applicable for vector control in the continent [22]. Similarly, PermaNet 2.0® and Olyset® nets are the most widely used LLINs in Africa. The data in this study had been collected over a six month period, following initial application of the IRS treatments (Chapter IV),

which would translate to two applications per year, being comparable to plausible re-spraying rates for most IRS applications.

To examine whether combination of any of these LLINs with any of the IRS would lead to improved community-level epidemiological benefits relative to IRS alone or LLINs alone, we simulated two different situations, 1) where people are already using nets, so that IRS is considered the complementary intervention and 2) where people are already using IRS with untreated nets, so that LLINs are considered the complementary intervention. For each complementary intervention, we calculated the relative improvement in malaria transmission control, in terms of the fold reduction of residual transmission. This is equivalent to the reciprocal of the relative residual EIR used in previous publications [6, 8, 9] and was calculated by dividing the estimated community wide mean entomological inoculation rate (EIR) for situations with just the baseline intervention with the mean EIR for situations with baseline intervention combined with the complementary intervention.

Results

The two most important results from this *in-silico* assessment were that: 1) combining LLINs with IRS does not always result in improved community level malaria transmission control relative to the use of either method alone, and 2) whereas introduction of LLINs into a community with pre-existing IRS generally results in improved malaria transmission control, introduction of IRS into communities with pre-existing LLIN use, is in most cases redundant except where the IRS compound is highly toxic to malaria mosquitoes.

Figures 2 and 3 show the simulation results in situations where IRS is the pre-existing intervention and where nets are the pre-existing intervention respectively. For

example, where there is no IRS but most people use intact untreated nets, replacing the untreated nets with two of the most common LLINs, Olyset® nets and PermaNet® nets can improve transmission control by 31% and 45% respectively, relative to the baseline. Similarly, where actellic IRS is already being combined with untreated nets, the two net types would provide an additional 14% and 35% transmission control respectively.

However, where IRS with DDT or lambda cyhalothrin is already in use with untreated nets, addition of these two LLIN types would be likely be redundant, except for an estimated 15% improvement when PermaNet® nets are combined with lambda cyhalothrin. Interestingly, these simulations show that in these same scenarios, replacing the untreated nets with Icon Life® net, would improve the impacts of IRS, providing 68%, 51%, 18% and 40% improvement in community wide transmission control when combined with no IRS, actellic, DDT or lambda cyhalothrin respectively (Figure 2).

On the contrary, situations where net coverage (of intact nets) is already high generally would not benefit from IRS, except where the IRS chemical is very highly toxic to the mosquito populations, such as actellic. For example, the simulations here show that introduction of DDT based IRS in such scenarios would either be redundant (when combined with untreated nets) or even worse, reduce the existing potential of transmission control if the pre-existing intervention were Olyset®, PermaNet® or Icon Life® nets. Similarly, addition of lambda cyhalothrin IRS would be redundant in places where most people already use any of the three LLINs, but the same IRS would result in marginal improvement where the pre-existing net coverage was with untreated nets. Actellic, the only one of the three IRS compounds that seems likely to provide additional benefits, is estimated to improve transmission control by 42%,

24%, 32% and 28% where the pre-existing intervention is untreated nets, Olyset[®], PermaNet[®], or Icon Life[®] nets, respectively (Figure 3).

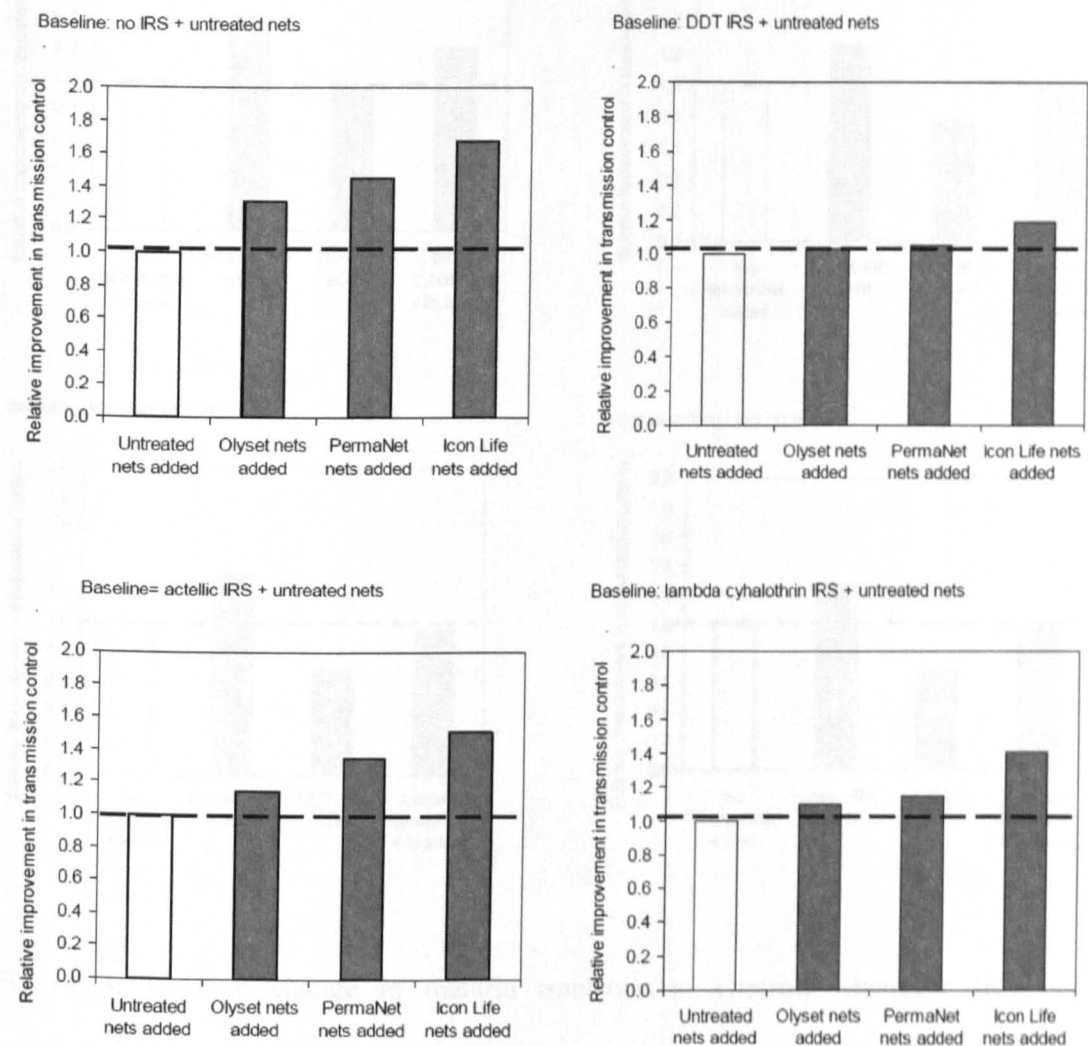
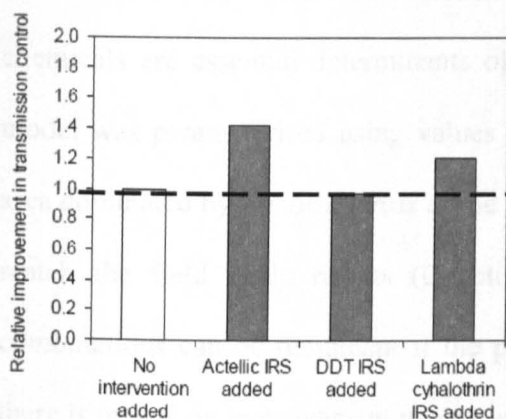
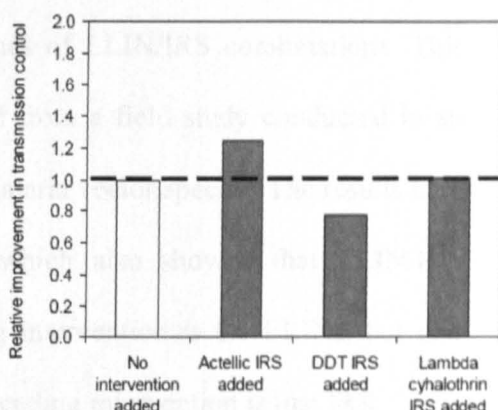


Figure 2: Relative change in malaria transmission control, whenever LLINs are introduced into communities with pre-existing high coverage of IRS and untreated nets. Values on the Y-axis can also be interpreted as the estimated ‘fold’ increase in transmission control relative to the respective baselines

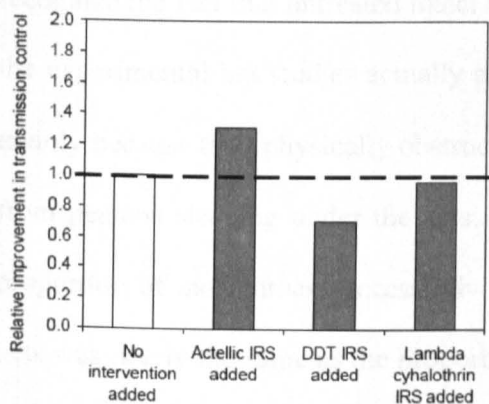
Baseline: untreated nets only



Baseline: Olyset nets alone



Baseline: PermaNet nets alone



Baseline: Icon Life nets alone

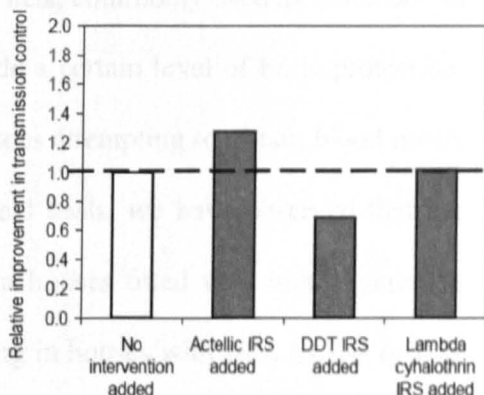


Figure 3: Relative change in malaria transmission control, whenever IRS is introduced into communities with pre-existing high coverage of nets. Values on the Y-axis can also be interpreted as the estimated ‘fold’ increase in transmission control relative to the respective baselines

Discussion

This work clearly shows that characteristics of the different LLIN types and IRS chemicals are essential determinants of outcomes of LLIN/IRS combinations. This model was parameterised using values obtained from a field study conducted in an area dominated by *An. arabiensis* as the major malaria vector species. The results here match the field study results (Chapter IV), which also showed that LLIN/IRS combinations can be redundant if the preceding intervention is the LLINs, but that there is mostly an improvement wherever the preceding intervention is just IRS.

Unlike all previous versions, these latest modifications of the model used here recognize the fact that untreated intact mosquito nets, commonly used as ‘controls’ in the experimental hut studies actually also provide a certain level of basic protection, mainly because they physically obstruct mosquitoes attempting to obtain blood meals from persons sleeping under the nets. In our field trials, we have observed that the proportion of mosquitoes successfully feeding in houses fitted with intact untreated nets was nearly the same as the proportion feeding in houses with IRS, LLINs or IRS and LLINs together (Chapter IV). This was despite the fact that other entomological measurements such as total mosquito catches inside the huts, proportion of these mosquitoes that died within 24 hours, and time of night when mosquitoes exited huts were all affected by introduction of an insecticidal application as opposed to untreated nets into the experimental huts (Chapter IV). This adaptation thus allows us to include values from most current hut studies, where controls actually consist of huts with human volunteers sleeping under untreated bed nets, as a basic protection.

The simulations presented here are only those with *An. arabiensis* as the major vector. We expect that some of the minor improvements such as those that are seen when lambda cyhalothrin is added onto houses with various LLINs, could be

significantly improved if the main target species was *An. gambiae* s.s. or *An. funestus* mosquitoes, which predominantly feed and rest indoors, and which are therefore more amenable to control using LLINs and IRS [14, 23] or if LLINs were old and torn and thus offered higher probability of mosquitoes obtaining a blood meal and resting indoors. Moreover, we have considered only a selected number of LLINs and IRS compounds. Two of these LLINs, Olyset® nets and PermaNet® nets are among the most common LLIN types currently being used in Africa. Similarly, actellic, DDT and lambda cyhalothrin are all approved by WHO for IRS, the latter two being among the most common.

The results therefore have a significant bearing on LLIN/IRS combination practices in Africa, especially as the malaria epidemiological picture is gradually changing and as *An. arabiensis* becomes the predominant vector in many areas of East Africa [16, 18, 24]. This work assumes that all other important determinants of LLIN/IRS effectiveness are optimal, for example that the IRS is sprayed regularly twice each year and that the bed nets remain intact and are used consistently throughout the year. Yet, these simulations still reveal many conditions under which combinations of the two interventions would double the expected outcome. It is expected that from a practical point of view, there would be even greater benefits of such combinations, given other factors such as the inability to ensure that nets remain intact and are used consistently, but also the rapid decay of some IRS compounds coupled with inconsistent re-spraying programs. In such cases, LLINs for example would be expected to extend the temporal insecticide coverage even after the IRS is decayed, while IRS on the other hand would be expected to confer additional protection to people using torn nets or people not consistently sleeping under their nets [1]. Both of these possibilities are not captured in the current simulations,

meaning that it is possible that the improvements observed here slightly underestimate the real value of LLIN-IRS combinations. Nevertheless, given that the best possible relative improvement is less than 1.5 in most of the common possibilities, the decision to implement LLIN/IRS combinations must be more carefully evaluated on the basis of available resources, giving preference to people who are already not covered by either intervention, and also ensuring that the best candidate insecticide is selected.

The most recent version of the model, used here, can be considered to be the one that best represents malaria transmission processes and how each stage can be affected by various indoor interventions. In addition to the latest division of diversion processes, which allowed for incorporation of effects of untreated mosquito nets, the subdivision of mortality processes is another essential characteristic for modelling of insecticidal interventions. This subdivision of mortality probabilities can be useful when modelling interventions that are specifically known to be fast acting, i.e. those that can kill mosquitoes immediately on attack, versus those that are specifically known to be slow acting, i.e. those that exhibit delayed toxicity to mosquitoes, e.g. fungal bio-agents [25] or insecticides such as chlorfenapyr [26, 27]. Moreover, where fed mosquitoes remain indoors and rest on walls, it is likely that interventions such as IRS would elicit most of the post exposure mortality. This has been shown in many experimental hut studies [1, 27], including those conducted to evaluate effects of DDT, where most of the mosquitoes found dead on the floor each morning were those that had taken blood the previous night [28]. In epidemiological perspective, interventions that kill mosquitoes after feeding confer mainly community level protection as opposed to personal level protection. However, interventions that kill prior to feeding also confer high levels of personal protection to users. Therefore, for purposes of simulating household level effects of combined interventions consisting

of both IRS and nets, it is possible to distinguish between these effects (μ_{pre} and μ_{post}) for purposes of estimating individual or household level exposure to transmission. This could particularly be desirable in situations where the proportion of mosquitoes that succeed in taking blood meals is high despite net use.

One possible limitation of these simulations is that they are based on the assumptions that the nets and the IRS are used the best way possible. For example, we have used the data from our experimental hut studies where volunteers always used the nets consistently, and also where all the nets were new and not torn. As a result, we observed that a very small proportion of mosquitoes were successfully obtaining blood meals. In practice, nets may often get torn, thereby increasing the likelihood that mosquitoes obtain blood meals from the human volunteers in the experimental huts. This would in effect lower the protective efficacy of the nets. Therefore, in order to actually achieve this simulated potential, all LLINs would need to be maintained in an intact insecticidal state, possibly by replacing the nets every one or two years.

Conclusion

We conclude that LLIN/IRS combinations can result in improved control or they can be redundant depending on what the pre-existing intervention is. Considering the most common LLIN types and IRS chemicals, malaria transmission control is enhanced when LLINs are added onto some IRS compounds such as actellic and lambda cyhalothrin, but not the irritant and less toxic IRS compounds such as DDT. However, transmission control is mainly redundant or even worsened when certain IRS treatments such as lambda cyhalothrin or are added onto households using the common pyrethroid net types, but at the same time, addition of highly toxic IRS compounds such as actellic can improve upon the potential of transmission control

relative to just the LLINs alone. Therefore it can be said that where IRS is the pre-existing intervention, at least untreated nets, but preferably the LLINs should be introduced and this would enhance the community wide transmission control. However, where LLINs are the pre-existing intervention, addition of IRS does not provide any additional benefit unless the IRS chemical is highly toxic and non-deterrent.

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Conflicts of interest

None

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Chapter IX

Summary and general discussion of the results

Preamble

In the beginning of this thesis, it was emphasized that because of their exceptional effectiveness, long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have now become the primary interventions against mosquitoes that transmit malaria. The two methods are widely associated with most of the recent successes in malaria control [1-4], a situation which has resulted in a drive by the international community to scale up these interventions in malaria endemic communities [5]. Evidence gathered by WHO shows a rapid shift from the use of ordinary insecticide nets conventionally treated by hand, to the use of LLINs, and also a phenomenal increase in coverage with both ITNs and IRS [3, 4, 6, 7].

Amid these trends, there are growing concerns that significant challenges still face insecticide-based malaria vector control [4]. The most important of these is undoubtedly the rise of insecticide resistance among vector populations [8, 9], but other issues including behavioural adaptation of malaria vectors [10-12] and its overlap with human behaviour [13, 14], changing compositions of the vector populations [11, 15, 16] and difficulties associated with maintaining protective efficacy of LLINs and IRS for extended periods of time under user conditions [4], are also exerting significant difficulties. Many malaria experts have already pointed out that these two methods in isolation may not be sufficient to achieve the current goal of

malaria elimination in many parts of Africa, thus they point to the need for integrated strategies and new vector control tools [17, 18]. The need to preserve and optimize the effectiveness of LLINs and IRS is therefore more urgent than ever [19, 20].

An international consultative group constituted under the hospices of the Bill and Melinda Gates Foundation recently asserted that the vector control focus points and tools required for sustained malaria control and for eradication should include: 1) new effective insecticides for LLINs and/or IRS, 2) appropriate integrated vector management, and 3) novel approaches to reduce high vectorial capacity of the major malaria mosquitoes [19]. More recently, a WHO consultative meeting to debate the technical basis for action against insecticide resistance in malaria mosquitoes [20], endorsed the following as potential means of preserving current insecticidal interventions: 1) rotation of insecticides, where different classes of insecticides are used in the same area but at different times alternately, 2) mosaics, where different insecticides are used at the same time but in different spaces, 3) mixtures, where at least two insecticides are co-formulated in the same dispensing mechanism and applied at the same time, and 4) combination of interventions, which involves using the different insecticide classes on different surfaces e.g. nets and walls, but inside the same house.

This last strategy has already been widely applied in a wide range of epidemiological scenarios, despite inconclusive evidence on whether indeed it achieves the desired health benefits [21]. What is clear is that even as the world seeks new tools to complement IRS and LLINs, these two methods themselves remain the most preferred [3, 5], and there is plenty of evidence to suggest that high coverage of households with LLINs and/or IRS are undoubtedly the most effective options available to control malaria in high transmission areas of sub-Saharan Africa [17, 22,

23]. Their application must therefore be optimised through evidence based decision-making processes, not only to preserve the accrued benefits, but essentially to ensure cost effectiveness, especially when they are to be used together in the same communities, and in light of recent restrictions in Global Fund support for malaria control [24].

The work reported in this thesis has focused on simultaneous use of LLINs with IRS in the same households, a practice that is already widespread in several malaria endemic communities [20]. The research aimed to determine whether there is any added advantage in combining ITNs with IRS at household level and to recommend the most appropriate insecticides for combined use, in regions with pyrethroid susceptible *An. arabiensis* as the primary vector. A summary of the main findings is provided below.

Summary of the major outcomes of the research

An in-depth review on the modes of action of insecticides used for IRS and ITNs and the potential benefits of combining ITNs with IRS

Since both IRS and ITNs are insecticide-based and are both used inside houses, it was hypothesized at the beginning of this research that outcomes of their combination would depend on how the candidate active ingredients affect mosquitoes that enter or those that attempt to enter houses. An in-depth review was conducted to examine modes of action of insecticides commonly used in these interventions [21] and to identify any existing evidence suggesting that ITN/IRS combinations would confer greater protective benefits than either ITNs or IRS when used alone.

One of the main findings of the review was that enhanced household level protection can be achieved where ITNs and IRS applications have divergent yet complementary properties, e.g. highly deterrent IRS compounds coupled with highly toxic ITNs. It is expected that the IRS in this case would provide additional level of protection by deterring mosquitoes from entering houses where people use toxic bed nets. However, it was also noted that care should be taken to prevent the spread of insecticide resistance alleles, by ensuring that the same class or related classes of insecticide are not used on the nets and walls [20, 21]. This situation may therefore present a certain level of dilemma to vector control experts. For instance, there is a large amount of evidence from previous experimental hut evaluations of ITN and IRS, which indicate that among the commonly used IRS chemicals, DDT and pyrethroids such as lambda cyhalothrin and deltamethrin are the ones, which can elicit significant deterrent effects on mosquitoes entering houses [21, 25, 26], yet it is also known that cross-resistance can occur readily between pyrethroids and DDT [27]. It should be noted however, that in the studies reported in this thesis, the deterrent effects were not as much apparent as in the previous studies (Chapter IV).

Nevertheless, the above argument also means that despite the fact that DDT and pyrethroids may have other beneficial properties such as toxicity, it would not be appropriate to use them together with today's pyrethroid treated LLINs, considering the increased risk of spread of insecticide resistance. In fact, WHO has now expressly suggested that pyrethroids should never be used for IRS where LLINs are already being used, arguing that such an excessive insecticide pressure would accelerate the proliferation of insecticide resistance alleles in communities [20]. The conclusion of our review regarding this dilemma was therefore that while maintaining a focus on the need for divergent and complementary modes of action, ITN and IRS products should

always be of different insecticide classes, e.g. pyrethroid-based nets combined with carbamate or organophosphate-based IRS [21], which is in line with current WHO guidelines [20].

If these results are put in the context of community wide protection, then it is also essential to consider other factors such as proportion of people covered by the interventions and the behaviour of vector species in a given area. It is reasonable to believe that IRS or LLIN interventions that do not kill many mosquitoes but instead deter a large proportion from reaching the persons inside the houses would be just as effective as highly toxic interventions provided that a large proportion of the people living in those communities are covered by the interventions. Other than simply diverting mosquitoes from one potential human host to another, high coverage would create a situation where mosquitoes are perpetually denied access to blood meals and are forced to host-seeking for prolonged periods of time; thus reducing their lifetime fecundity through increasing the length of the oviposition cycle [28]. This is most likely to happen where the predominant vector species are not opportunistic, but feed mainly on humans indoors, in which case the final outcome could include near-complete or complete disappearance of the species [29, 30].

Another important finding of the review work was that there are multiple reasons why LLINs are combined with IRS, and therefore any criteria for assessing these combinations should take this into consideration. It was noted that in most cases, the combination strategy is recommended as a way to accelerate malaria control in high transmission areas [22, 23, 31, 32], where the use of IRS alone or ITNs alone may not be sufficient to reduce the transmission intensity to acceptable levels [17, 19], but where transmission must be reduced to near-undetectable levels before any significant declines in malaria prevalence can be achieved [19, 33]. Yet, even in these

situations, the combination of methods, despite being implemented, has not been categorically proven to have any additional effects on malaria transmission relative to either method used in isolation [21]. The second reason for combining LLINs with IRS is to ensure long-term household level protection where one of the interventions can rapidly become weakened e.g. using LLINs where IRS activity decays after a short time [34-36]. This is particularly important given that most IRS compounds in use today do not retain their efficacy beyond a few months [37], and multiple spray rounds per year may not always be logistically or economically feasible low income countries [22]. The temporal benefits of adding LLINs into houses with more short-lived IRS treatments are therefore obvious and the practice should be encouraged. Based on results from our own field study, which is described in detail in Chapter IV, we expressly suggest that even where insecticidal nets are unavailable, IRS treatments must be supplemented with at least untreated nets, rather than being used alone.

The third reason for combining LLINs with IRS is more concerned with community level protection rather than individual or household level protection. We noted that combinations may also be used to increase overall coverage with vector control where complete coverage with only one of the interventions is not readily feasible throughout all endemic communities due to either logistical or cultural factors. It may therefore be worthwhile that when the additional interventions are introduced, priority is given to households or communities that are not already covered or being targeted with the pre-existing interventions, so as to expand the overall community-wide coverage.

The fourth reason for combination of LLINs with IRS was, as already mentioned above, to mitigate insecticide resistance. Even though there is not yet any direct evidence that combining different insecticide classes would slow the spread of

insecticide resistance, there is evidence of higher mortality rates among resistant mosquitoes exposed to multiple insecticides in combinations, mosaics or mixtures [38-40]. It is expected that using IRS and LLINs with differing insecticides e.g. a pyrethroid-treated LLIN and the organophosphate or carbamate IRS may therefore slow the spread of insecticide resistance [20]. As LLINs and IRS continue to be scaled up in malaria endemic areas, the threat of insecticide resistance also increases thus management of gene mutations to the common classes of insecticides (pyrethroids, organochlorides, carbamates and organophosphates) need to be emphasised. Though the in-depth review focused primarily on data from sites where no insecticide resistance had been reported, it is reasonable to assume from the limited available data that where insecticides of different modes of action are used, mosquitoes that are resistant to one of the insecticides could still be killed by the other insecticide, thus delaying any selection for resistant mutants among the mosquito populations [20]. The actual possibility that combinations can continue to protect against resistant vectors has now been examined in experimental hut studies in west Africa, with favourable results suggesting that indeed IRS/LLIN combinations with divergent insecticide classes can be used against insecticide resistant vectors [38].

At the end of the review, it was suggested that controlled basic and operational research, complemented with mathematical modelling, should be conducted to evaluate IRS/ITN combinations in comparison to IRS alone or ITNs alone. This would clarify whether LLIN/IRS combinations are indeed synergistic and will therefore enable informed decision-making to optimize the effectiveness of the two interventions. Relevant focal points that the proposed research should focus on were identified as follows: 1) synergy and redundancy as measured directly, based on effects of IRS insecticides and LLINs on malaria mosquitoes, 2) choice of appropriate

insecticides to use in the LLIN/IRS combinations, 3) epidemiological and operational determinants of successful LLIN/IRS combination, 4) relevance of LLIN/IRS combinations as a tool to manage insecticide resistance, and 5) cost-effectiveness of the LLIN/IRS combination as a strategy. Other than the question on cost effectiveness, the other key items have all been addressed in this thesis to varying extents, through a combination of experimental hut studies and mathematical modelling.

An improved experimental hut assay for evaluation of LLINs, IRS insecticides and their combinations for malaria vector control

The easiest way to test indoor vector control interventions would be to introduce them into human occupied houses and observe how they affect malaria vectors that enter in those houses. However, in Chapter III of this thesis we highlighted the fact that differences between individual human houses can confound results of such studies, indicating the need to develop standardised systems that mimic conditions in human occupied houses. Such systems can then allow LLINs, IRS or both methods to be rigorously tested in an experimentally controlled manner.

The most obvious option to adopt for the field studies reported here was to use one of the many experimental hut designs previously developed and used by mosquito researchers in Africa [41]. However, a careful review of the existing designs and the associated research data revealed that many of the huts had a number of limitations and therefore needed to be improved so as to ensure better representativeness of local houses most commonly found in rural Tanzania. Most notable disadvantages included: 1) the fact that some of the designs, e.g. the east African veranda trap huts [42] do not allow for sampling of mosquitoes on all sides of huts during the same

night, 2) the likelihood of live mosquitoes flying out of the experimental huts, through open spaces on the huts, such that those mosquitoes that remain inside are mainly the dead ones, a situation which would cause an over-estimation of proportions of mosquitoes killed by any candidate insecticides being tested, 3) difficulties of cleaning and decontaminating the huts when a new insecticide is to be tested, especially where multiple studies are aimed to be conducted consecutively using the same experimental huts, and 4) the generally small size of the experimental huts, which can misrepresent the ratio of treated surface to volume of air and airflow present in local houses.

This last point is particularly important when considering the modes of action of many vector control insecticides, for which mode of action (excito-repellence versus toxicity) [43] and efficacy is strongly dose-dependent. Therefore, as a first step towards the field study, we developed a modified experimental hut design, *the Ifakara Experimental Huts*, and successfully validated its design in rural Tanzania [44]. Through a series of baseline evaluations, some of which were conducted by spraying botanical mosquito repellent, para-methane 3, 8 diol, (PMD), we ascertained that this design can indeed be used for assessing effects of indoor interventions including LLINs and IRS, or their combinations of disease transmitting mosquitoes, including the malaria vectors, *An. arabiensis* and *An. funestus* [44].

Whereas huts such as these could possibly be used in many different ways with different mosquito trapping methods, the specific experimental procedures that we applied when using these huts, were those that ensured accurate representation of the behaviour of disease transmitting mosquitoes in and around human occupied houses. Some of the key characteristics of these new experimental huts include the following: 1) interception traps fitted onto eave spaces and windows, and which can

be used to sample mosquitoes on all four sides of the huts, 2) use of eave *baffles* (panels that direct mosquito movement) to control exit of live mosquitoes through the eave spaces, such that live mosquitoes that enter the huts do not escape through the open eave spaces, but exit into interception traps, 3) use of replaceable wall panels and ceilings, which allow safe insecticide disposal and reuse of the huts to test different insecticides in successive periods, 4) the kit format of the huts allowing portability and 5) an improved suite of mosquito collection procedures designed to maximise data quality [44].

While we recognize that all no experimental huts can capture the full variability in conditions and designs of local houses, the Ifakara experimental huts provide the much needed improvements relative to many of the previous hut designs, which had clearly not achieved the goal of matching local houses. To illustrate this, one might consider the examples of the East Africa veranda trap huts [42], which are very small and are unlikely to have similar airflow as in local residential houses, notwithstanding the fact that this parameter has not been measured even in our experimental huts. Similarly, the West African huts such as those used in Benin [45], allow mosquitoes to enter huts via very small slits on the sides, thus restricting the natural entry pattern and also adjusting the airflow in the huts. But improvements in the new hut designs were not only on the physical design. Instead, even the way mosquitoes were collected in many of the previous huts has been improved [44]. For example, collections in previous hut studies, which often involved retaining trapped mosquitoes in close proximity to the huts until morning, was considered to not necessarily represent the natural behaviour patterns of mosquitoes, especially where users are protected with nets.

The Ifakara huts and the interception traps with which they are used, have incorporated improvements which allow for multiple mosquito collections each night, such that mosquitoes attempting to exit the huts are not unnecessarily detained in the huts, thus minimising chances of unrepresentative overexposure to insecticides. This problem of over-estimated mortality among vectors held close to experimental huts was identified in Smith and Webley 1963, but was largely ignored to date [46]. The consistently lower insecticide-induced mortality identified among mosquitoes collected in these huts (Chapter IV) relative to studies of the same insecticides with other hut types [21, 25], may indicate that there may be an overestimation of insecticide toxicity in other experimental hut designs. In this regards, it is greatly encouraging that further collaborative studies with other insecticide test facilities, approved by WHO pesticide evaluation scheme, are already underway to investigate this potential confounder (Dr. Sarah Moore, Pers Comm) and to comparatively evaluate these new huts against different existing hut designs.

Though these huts were developed primarily to test LLINs, IRS or their combinations, we recognize that their utility goes way beyond this and that they can be applicable for several other studies. In our preliminary behavioural assays, for which results have been presented in Chapter III, the huts were used to assess the natural behaviour pattern of mosquitoes in the study area, where *An. arabiensis* was shown to prefer hut entry via eave spaces, but to exit mainly through windows [44]. Other than these, the huts are already being used in Tanzania, Kenya, Zambia and Benin for various studies, including: 1) evaluation of different LLINs and IRS chemicals (Chapters IV and V), 2) house screening against human biting mosquitoes [47], 3) mosquito area repellents (N'Guessan *et al* unpublished; Ogoma *et al*

Unpublished), 4) synthetic mosquito attractants [48] and 5) mosquito killing fungal pathogens [49].

Considering the need to integrate results from different tests conducted on universally applicable vector control tools, one relevant step to take would be to make a decision on how to interpret results originating from studies conducted using different experimental hut designs. As noted in Chapter III, despite its improved characteristics, we cannot at this stage propose the Ifakara experimental hut design as a replacement of any existing hut designs. Instead, we concluded by strongly recommending that prospective users should independently assess the utility of these new huts in their respective situations before using them. Nevertheless, the entomological procedures described here provide a framework that may also be modified to more accurately match intended research purposes and to better evaluate effects candidate interventions being tested in different places or hut types.

Characterization of household level effects of LLINs and IRS when the two methods are used alone or in combination.

The overall objective of this research was to determine whether protective efficacy of LLINs combined with IRS, would be greater than that of either the LLINs alone or IRS when used alone. This is an all important question that researchers have recently began to consider in great depth, partly because of the realization that there is an urgent need to preserve and enhance the protective benefits being accrued with the two methods [19, 20, 50]. For many years, WHO recommended that insecticidal nets should be combined with IRS to control malaria especially in holoendemic and hyperendemic communities [5, 22, 23]. These recommendations were based mainly on theoretical evidence rather than empirical field evidence which has remained

largely inconclusive [21, 51]. In 2009, Kleinschmidt *et al* completed a review of various studies in Africa, where LLINs and IRS had been used together, and concluded that there were mixed results, some showing synergy while others showing redundancy [51]. As noted earlier in this discussion, we also examined results from a number of research studies and mathematical simulations previously conducted by different experts, and concluded that while there are several theoretical justifications for combining LLINs with IRS, it was necessary to urgently conduct field studies to conclusively answer this question [21].

Perhaps therefore, the most useful outcome of this thesis work has been this field evidence, which becomes one of the earliest reports of research into LLIN/IRS combinations in Africa. The only other published study attempting to address this question is the greatly successful study conducted by Ngufor *et al* in Benin [38], which considered combinations of pyrethroid based LLINs with a pyrole insecticide, chlorfenapyr, for use against insecticide resistant malaria mosquitoes.

In our studies, we evaluated not one, but three different types of LLINs and also three different IRS insecticide classes. The LLINs were Olyset® (a permethrin impregnated bed net), PermaNet® (a deltamethrin coated bed net) and Icon Life® (deltamethrin impregnated bed net), while the three IRS chemicals included an emulsified concentrate of actellic (an organophosphate), wettable powdered DDT (an organochloride) and a capsule suspension of lambda cyhalothrin (a synthetic pyrethroid). The idea was to consider a widely representative array of chemicals currently approved by WHO for malaria vector control [37], which currently include 12 different insecticides for IRS, 6 for use on bed nets and 2 insecticides for both nets and IRS [52]. The IRS compounds used here, and all the LLINs, except Icon Life®, were among those approved [52], and were selected as being representative of

insecticide classes most commonly used in Africa today. Moreover, available evidence suggests that many of these insecticides can elicit distinct effects on mosquitoes, but also that different formulations of same insecticides in net brands can confer varying levels of protection [21]. Therefore, in the process of assessing synergy between LLINs and IRS, this study not only determined which of the candidate insecticides would make the most effective combinations at household level, but it also characterised the individual insecticidal treatments based on their effects on malaria mosquitoes. This means that the results obtained here can be considered the most comprehensive set of empirical data available on efficacy of LLIN/IRS combinations for malaria vector control at household level.

These studies clearly showed that all net types, including non-insecticidal nets, if used consistently and maintained in an intact state, can provide near absolute protection from mosquito bites (> 99% feeding inhibition), regardless of whether they were used in combination with any IRS or not. Addition of LLINs into huts with IRS treatments can provide additional protection, by inhibiting feeding and by causing excess mortality especially where the deltamethrin treated nets, e.g. PermaNet® or Icon Life® are used. Similar to findings by many previous researchers, which were earlier reviewed here [21] and are reported in Chapter II, we found in these field trials that deterrence is generally not an effective property of LLINs.

For this reason, one of the key take home messages put forth in Chapter IV was as follows: for improved protection at household level, and in order to ensure that fewer mosquitoes enter houses, LLINs may best be combined with IRS treatments that have some deterrent effects on mosquitoes attempting to enter households. In our studies, we observed, albeit only in the first spray round, that addition of IRS using DDT deterred more mosquitoes from entering the huts already having LLINs, but did

not increase proportional mortality. This property of DDT has of course also been shown in many previous studies [26, 53]. The challenge therefore is that such treatments must be delivered as part of high coverage community level control programmes so that any mosquitoes that are deterred from users do not find any accessible non users nearby. Otherwise highly toxic IRS compounds should be preferable to maximise community level benefits.

An interesting explanation has been advanced by Yakob *et al* [54], who modelled potential effects of combining DDT with LLINs. In simulations of houses fitted with these applications, they observed a high degree of antagonism between the two, and explained this as a biological phenomenon arising from interference between modes of action of the insecticidal applications. That is to say IRS treatments such as DDT, which deter mosquitoes from entering houses [26, 55], would reduce the frequency with which LLINs used inside those sprayed houses are contacted by mosquitoes. Similarly, the nets would reduce rates of blood feeding, meaning that fewer mosquitoes would need to rest on the sprayed walls. In this case therefore, even though persons sleeping inside those specific houses may experience a large reduction in mosquito house entry and would be protected effectively, the overall community protection would be lower given the high survival rates of mosquitoes that do not contact insecticides on LLINs and walls. This is surely an elegant explanation, but it does not appreciate the fact that blanket community level coverage, even with these DDT plus LLINs, would create situations where vector mosquitoes are constantly denied access to human hosts and therefore transmission would inevitably reduce, as has been shown in practice historically [29, 53, 56]. Therefore the stronger argument against combining DDT with current LLINs is in our view, the risk of increased

insecticide pressure, potentially giving rise to rapid proliferation of resistance in the vector population.

In Chapter IV, it was also shown that IRS with actellic significantly increases proportional mortality relative to LLINs alone, but that this chemical does not induce any deterrence. This kind of combination, by killing a larger number of mosquitoes can ensure greater community benefits as well as significant household level protection provided that the LLINs used in the house are intact and are consistently used, so as to minimize feeding. This argument was corroborated in Chapter VIII where the huts data were fitted into deterministic mathematical models that simulate malaria transmission in whole communities with different epidemiological characteristics.

It is necessary to focus all these findings towards our most important question, i.e. whether there are any added protective benefits achievable at household level by using LLINs together with IRS instead of either method used on its own. In this respect, the main conclusions of this thesis are as follows: first, there are minimal additional protective benefits to be gained from adding IRS with DDT or lambda cyhalothrin into houses where people already correctly and consistently use existing LLINs, even if the vectors in the area are still susceptible to the insecticides. Second, given the range of insecticides currently available for malaria control, combining pyrethroid based LLINs with IRS would be most effective if the IRS of choice was a highly toxic chemical, e.g. actellic. It is necessary to add here that in line with current expert arguments on how to deal with the challenge of insecticide resistance [20], we have also stated in Chapter IV, that such combinations of pyrethroid based LLINs with highly toxic organophosphates have an added advantage of applicability as a measure for insecticide resistance management.

Third, IRS should preferably not be used alone, regardless of the actual IRS insecticide, but should instead be supplemented with at least untreated nets even where insecticidal nets are not readily available. In other words, where the pre-existing intervention is IRS, there is a strong need for nets to enhance individual personal protection both immediately and also for a prolonged period of time even after the IRS has decayed, i.e. far in excess of the 3-4 month life of most IRS treatments [52]. In places where the pre-existing intervention is LLINs, a reasonable decision can be made regarding the need for additional IRS considering the need for additional personal and community protection, dependent on the longevity and consistent use of the LLINs, malaria epidemiology and the need for insecticide resistance management. Lastly, where resources are limited, focus should be that everyone in a malaria risk area uses an LLIN consistently, instead of trying to combine LLINs with IRS. Nevertheless, we also recognize that in situations where it is not possible to provide everyone with LLINs or where the LLINs cannot be maintained in an intact state or used consistently due to social factors [57], as well as in epidemic, elimination or emergency situations, carefully timed IRS with highly toxic insecticides should be added to provide the necessary communal protection by killing excess malaria mosquitoes.

With these conclusive statements, it should be noted that our evaluations were conducted in the best possible conditions, where the interventions were used correctly and consistently. Moreover, results obtained from our complementary tests of bio-efficacy and residual activity of these compounds (Chapter V) suggest a fairly rapid decay of the IRS compounds from treated surfaces, after just a few months of spraying. As such we strongly recommend that the use of LLINs should be prioritized and considered as the basic minimum regardless of availability of IRS. All necessary

resources and logistical support for LLIN distribution should thus continue to be actively sought and provided. On the other hand, the use of IRS in areas Africa with *An. arabiensis* as the primary vector should be considered secondary to LLINs, to be implemented only where there are adequate financial resources and logistical feasibility.

Characterization of bio-efficacies and residual activity of insecticides commonly used for LLINs and IRS

In the history of malaria vector control, decisions to use IRS, LLINs or the two methods together, have often been guided by three important determinants: 1) an understanding of local epidemiological conditions, 2) logistical and operational requirements and 3) known protective efficacies of the interventions [21]. We now know that the third factor in this equation, i.e., protective efficacy, is itself dependent upon the behaviour of local mosquito populations [10], and the susceptibility of these vectors to insecticides used for IRS or on the nets [27]. For mosquitoes to be affected by any insecticidal application, they must come into contact with the insecticides or its fumes, and must also not harbour any resistance characteristics, which would otherwise limit the ability of the insecticide to kill the vectors.

When insecticidal interventions are first applied, their efficacy immediately begins to deteriorate, and eventually, the concentrations of active ingredients become so weak that the mosquitoes are not affected any more. It is therefore essential to have a comprehensive understanding of the rates of these decays and to know at what stage, individual insecticidal applications fail to be effective. This way, efficacies of the selected treatments can then be boosted, e.g. by re-spraying, in the case of IRS, or by introducing a complementary intervention. We pointed out earlier that the need for

temporal protection can itself be a justification for combining LLINs with IRS. That is to say, for example, that LLINs would provide extended protection when the IRS has decayed to an ineffectual dose. Similarly, IRS would provide continued community protection when LLINs have been torn or during those times when the net users are actually out of their nets and mosquitoes are active. Other than the understanding of temporal changes in efficacy of actual interventions, such assessments can also constitute an early exploration of possible insecticide resistance mechanisms, that would otherwise hinder effectiveness of insecticide based interventions [58].

In this respect therefore, another important outcome of this PhD study was the longitudinal characterization of both bio-efficacies and residual activity of insecticides used for IRS and LLINs. The residual activity tests included bioassays performed using standard WHO cones and wire balls on LLINs and sprayed hut surfaces, while the bio-efficacy tests included standard WHO-susceptibility tests performed using diagnostic concentrations of candidate insecticides, against wild mosquitoes collected in the study area. Molecular analyses were also conducted to detect any *kdr*-resistance alleles if any, in the malaria vector population. This characterization exercise was primarily designed to complement our efforts to assess synergies and redundancies in household level protection, when IRS is combined with LLINs. The data generated proved to be immensely useful in analysing findings from the latter study, and must therefore be considered as such. For example, the confirmation through the bioassay tests that mosquitoes in this study village were still 100% susceptible to DDT, and the failure of our molecular assays to detect any *kdr*-resistance alleles in local vector populations, means that the findings from experimental huts, in which IRS with DDT did not elicit high mortalities on *An. arabiensis* could not be associated with resistance to this class of insecticides. Instead, this phenomenon was judged to be due

to the behaviour of the vectors, which caused low contact rates with sprayed surfaces inside the huts, but also the improved design of our experimental huts, which ensured that mosquitoes were not unnecessarily over-exposed to the treatments through prolonged retention in the huts, and that all mosquitoes that entered the huts were collected, as opposed to collecting only a subpopulation of those that entered as in other early hut designs [44].

Other interesting findings in this regard included the observation that while all candidate IRS formulations (DDT wettable powder, lambda cyhalothrin capsule suspension and actellic emulsified concentrate), were highly effective during the first month after spraying (killing $\geq 85\%$ of mosquitoes exposed in cone bioassays) these treatments rapidly decayed, losing most activity within 1-3 months. Very surprising indeed was the finding that the tested LLINs (Olyset[®], PermaNet[®] and Icon Life[®] nets), also lost insecticidal efficacy, in some cases by greater than 50% in just six months. This might indicate the importance of regularly washing LLINs, which was not done in this study, but which is necessary to ensure regeneration of net activity [59]. Finally, in addition to the aforementioned finding that malaria vectors in this study area were fully susceptible to DDT and that no knock-down resistance gene mutations were detected in the population, we observed a weakening susceptibility (mortality in the range of 90.2% to 95.8%) to lambda cyhalothrin, permethrin and deltamethrin, which signifies the need to be vigilant against pyrethroid resistance in the area. Perhaps it is important to note that an independent survey also already reported that *An. gambiae* s.l population in this area was suspected to be in the early stages of developing insecticide resistance [60].

Given these results, we have concluded that existing pyrethroid-based LLINs remain the most efficacious insecticidal intervention against malaria vectors in the

area. However, if we consider the other results reported in Chapter IV, then it is appropriate to say that given the rapid decay of insecticidal activity from the IRS sprayed mud surfaces, and also the possibility that unfed mosquitoes might not rest long enough on treated surfaces to pick up lethal doses of the insecticides, IRS if used here must always be complemented with LLINs, to achieve significant impact. These results effectively reinforce our justification for adding LLINs where IRS is the pre-existing intervention, so as to provide the necessary continued protection even when the IRS decays. There is however, no evidence to support introduction of IRS into houses where LLINs are already being used. Finally, the signs of tolerance to pyrethroids in the area suggest not only the need for improved vigilance against resistance, but also the need for a strong caution against using of pyrethroids for IRS in this area. Because pyrethroid-DDT cross-resistance is increasingly becoming a major challenge to insecticide based malaria interventions in Africa [8, 61], this caution should inevitably extend to the use of DDT for IRS in the area.

Simulated community level effects of combining LLINs with IRS

The data generated from the field experiments described above, enabled us to assess the potential of LLIN/IRS combinations at household level, and as such, our ability to make inferences regarding community level protection were minimal. Besides, the experimental data came from only a single study village with fixed epidemiological characteristics. Therefore, to be able to make generalizable inferences, it was necessary to input this data into simulations that allow 'creation' of multiple epidemiological scenarios and thereafter an examination of synergies or redundancies when LLIN-IRS combinations are used at community level. Moreover, this would

also allow testing of multiple interventions by incorporating those characteristics measured during the experimental hut assays.

A three stage approach was taken to achieve the final simulation for this purpose. First, an existing deterministic model [28, 62], was adapted and modified to more accurately represent processes in the mosquito life cycle, that are relevant to malaria transmission, and to assess how these processes can be affected by different interventions used against the vectors. Once this was achieved, the simulations were first tested for situations where LLINs are combined not with IRS, but with an outdoor mosquito control strategy, odour baited mosquito traps [63]. Part of the reason for this approach was that whereas at this stage we were already having adequate data on odour baited mosquito traps, tested in the same study area [48, 64], no data was available for IRS as yet. Besides, our strategic view was to develop a model that would be applicable for evaluating many different vector control interventions, rather than just LLINs and IRS. This initial stage of modelling generated some convincing theoretical evidence on the potential of mosquito traps for malaria control and elimination in Africa [63]. For example, it was shown that traps baited with synthetic lures [48] and used at the rate of 20-130 devices per 1000 people would be as effective as at least 50% coverage LLINs, and that if combined with the nets, the intervention would potentially drive malaria transmission beyond thresholds necessary for malaria elimination, in several scenarios representative of Africa [63].

The second stage involved improvements and further testing of the model. One of the key changes introduced at this stage was the sub-classification of mosquito mortality events, such that it was possible to quantify the mortality that occurs after mosquitoes have already taken blood (as with IRS), versus that which occurs before blood meals (as with LLINs) [65]. In the original versions, we had assumed that all

the mortality events occurred before the mosquito was able to feed on the target host [63]. One major limitation of that approach was that it would not be possible to represent first acting interventions, i.e. those that kill mosquitoes as soon as they enter houses and make contact with the insecticidal surfaces, as opposed to slow acting interventions, which allow mosquitoes to take blood meals and possibly digest it before dying later.

The improved version of the model was tested by modelling effects of insecticides that primarily deter mosquitoes from humans versus those that are mainly toxic to malaria mosquitoes, these being the two main modes of action of common malaria vector control insecticides [65]. The working hypothesis in this simulation was that while deterrence enhances personal or household protection of LLINs and IRS, it may also attenuate communal protection if used at high coverage, or reverse communal protection if it diverts mosquitoes to non-users rather than killing them outright. In the sections above, examples have been described that are based on our own observations and reviews on DDT and pyrethroid-based LLINs. This improved model, therefore enabled a more accurate representation of the sequential interactions between deterrent and toxic actions of insecticides, and also accounted for the distinctive impacts of toxic activities that kill mosquitoes *before* or *after* they have fed on intervention users.

Similar to our predictions using DDT as an example, we observed here also that increasing deterrency also increases personal protection but consistently reduces communal protection, unless coverage is high and the mosquitoes have no alternative blood hosts other than humans, since deterrent sub-lethal exposure inevitably reduces the proportions of vectors that are subsequently exposed to higher lethal doses. If the high coverage as stipulated in current WHO guidelines [5] are achieved, purely toxic

products with no deterrence are predicted to generally provide superior protection to non-users and users, especially where vectors feed exclusively on humans and a substantial amount of transmission occurs outdoors. Remarkably, this would also happen if that product confers no personal protection, and only kills mosquitoes after they have fed, demonstrating the importance of driving down the absolute size of the vector population on lowering malaria transmission, which can be achieved far more easily through the use of highly toxic IRS combined with LLINs.

The final stage of this modelling (which is detailed in Chapter III) involved its application of the optimised model to examine what would happen if the candidate LLIN and IRS insecticides described in Chapters IV and V were used either alone or in combination. That is to say, would such combinations be synergistic or redundant, relative to use of either method alone? Given that the huts experiments were conducted without a pure negative control, but instead by using intact untreated bed nets as basic minimum protection for volunteers, we modified the model further to take into consideration the possibility that untreated nets also offer some basic protection, usually by directly preventing mosquito bites, and are therefore in themselves a viable purely deterrent intervention.

Even though this final model allows for creation of conditions with varying epidemiological characteristics, this objective was accomplished by simulating a closed community scenario where residents own cattle, and where the main malaria vector is *An. arabiensis*, an increasingly dominant vector species in Africa, and a vector which continues to present significant challenges to the control even with high LLINs and IRS use rate [11, 15, 17-19]. Moreover, we considered situations where either the LLINs or the IRS are the pre-existing interventions. Therefore, in order to examine redundancy or synergy, achieved by adding IRS or LLINs as the

complementary intervention, we calculated a relative improvement in malaria transmission control.

What was really interesting here was the close match between the findings of this simulation to what we had hypothesised based on the data generated from the experimental hut studies (Chapter IV) and also our review [21]. Specifically, these simulations also showed that whereas introduction of LLINs into communities with pre-existing IRS will generally improve transmission control, introduction of IRS into communities with pre-existing LLIN use, would be redundant unless the IRS compound is highly toxic to the malaria mosquitoes. It was shown clearly that malaria transmission control can be synergised when any of the currently available pyrethroid-based LLINs are added into houses sprayed with IRS compounds like actellic and lambda cyhalothrin, but not DDT, which as shown in the field study, tended to have lower toxicity but moderate deterrence on mosquitoes.

Nevertheless, the specific finding that DDT based combinations, even at high coverage do not cause synergy, matches the findings we obtained at the second stage of this modelling exercise [65], but does not match with our hypothesis of blanket protection as suggested in the beginning [21]. This can be attributed to two aspects of the modelling work: 1) the inclusion of cattle as an alternative blood host in these simulations and the description of the main vector, *An. arabiensis*, as being a vector that readily feeds on cattle, and can therefore survive and maintain transmission even when humans become unavailable indoors, and 2) the inclusion of a correction factor in the simulations to represent the fact that not all the malaria transmission that occurs indoors is preventable by the indoor interventions, meaning that transmission can continue to occur when the deterred mosquitoes contact the net users at times when they are not using their interventions [13, 63, 65].

Unlike all previous versions of the model [63, 65, 66], these last modifications of the model recognized the fact that untreated mosquito nets, commonly used as ‘controls’ in experimental hut studies actually also provide a basic level of protection, mainly because they physically obstruct mosquitoes attempting to obtain blood meals from persons sleeping under the nets. This was already very clearly observed in the hut study, where there was up to 99% protection from mosquito bites even in huts with untreated nets as the only intervention, i.e. same level of protection from bites as conferred by LLINs. Therefore, despite the fact that untreated nets may not have similar properties as toxicity and deterrence that are elicited by insecticidal interventions, they can be a viable option to consider as an addition to IRS. We have therefore suggested here that intact long lasting untreated nets (LLUN), used in rotation with LLINs or in combination with IRS, should be debated and tested further as a means for insecticide resistance management. It also highlights the importance of regular distribution campaigns to replace those nets that have become physically damaged.

General discussion of the results

Prior to this study, there was minimal field evidence to support LLIN-IRS combinations relative to the use of either method alone [21]. The available data was largely inconclusive and had been generated from a variety of field studies, in which either there were numerous confounding effects or there were no suitable experimental designs put in place to address this specific question [21, 51]. In the course of the research reported here, there have been at least two new field studies that have addressed this question, one of which assessed effects of a single combination of LLINs with one type of insecticide, against resistant mosquitoes in

Benin [38], and another which examined clinical outcomes of LLIN-IRS combinations in non-randomised prospective cohorts in Kenya [67].

Our study has extended this limited evidence base, providing the first set of field data by directly comparing house-hold level effects of multiple combinations of different IRS and LLIN types versus either the nets alone or the IRS alone. These house-hold level results have further been augmented by theoretical evidence of community-level effects, which were obtained from mathematical simulations of situations where the LLINs are combined with IRS. These two aspects of the study (the field experiments and the mathematical simulations) therefore present a greater picture of what is likely to happen both at the household level and at community level when any of the common IRS compounds are used together with any of the current LLINs. The study indicates very clearly that adding any of the two interventions onto the other can enhance protective efficacy, but also that there is need to carefully select the methods based on their modes of action, to achieve maximum benefits. Moreover, given the rapid decay of some of the insecticides from treated surfaces, the combinations necessary to confer some temporal overlap of protection, for example LLINs continuing to provide protection where IRS has decayed. Most importantly, the study has shown the greatest communal impact would be obtained if the IRS being used together with the LLINs were highly toxic to malaria mosquitoes.

Both LLINs and IRS, despite being the best available vector control measures are prone to certain limitations that may hinder their overall effectiveness for malaria control. For instance, LLINs when torn can lose some of their protective efficacy both against mosquito bites and potential malaria infection [68]. Moreover, net ownership is not always equivalent to proper net use [57], and in many cases the nets are not always used correctly and consistently by people who own them. In the same

regard, mosquitoes that bite early in the evening, usually before people go to bed may not be sufficiently targeted by the nets. As a result, the proportion of malaria transmission that nets can actually prevent is always lower than 1, and even in the best case scenarios, this proportion is not expected to exceed 0.9 outside experimental conditions [13, 14]. Similarly common IRS insecticides rapidly decay from sprayed surfaces and therefore can become ineffective after just a few months [52]. Besides, implementation of IRS is a resource intensive exercise. It often requires extensive planning for transport and storage of the chemicals to be used and for management of the spray teams during the campaigns. In some communities, not all homes are accessible to the spray teams, meaning that the desired coverage may not always be achieved. IRS may therefore not be suitable for every setting and is often implemented only in selected locations and during selected times of the year, in which case it is not always expected to provide protection all year round [22]. Lastly, both IRS and LLINs target mainly those mosquitoes that enter or those that attempt to enter human dwellings. Therefore, other than the accumulated communal benefits [1, 69], which result from the fact that these interventions also kill mosquitoes that come into contact with them, the two interventions are not always directly effective against vectors that bite humans outdoors or those that rest outdoors.

However, when the two interventions are combined, it is expected the users get enhanced protection both at household level but also at communal level, resulting from either the increased number of mosquitoes being killed by both IRS and LLINs, or the additional prevention of mosquito bites, that is afforded by the mosquito nets. The data presented in this thesis show that indeed the additional benefits obtainable from IRS-LLIN combinations occur mainly due to the excess killing effect and the direct protection against bites (Chapter IV). Mathematical simulations of control

scenarios where the different interventions are used either alone or in together also suggest that the most efficacious combinations would be those that consist of the current pyrethroid-based LLINs, used alongside highly toxic IRS compounds such as actellic (Chapter VIII). Since the outcomes of any such combinations significantly depend on the type of the candidate interventions, i.e. the active ingredients used on the nets or the IRS, careful pre-implementation assessment is required to select the most appropriate candidates for the combined strategy.

To some extent, the outcomes of this work may seem to be slightly biased in support of LLINs rather than IRS. We have suggest that LLIN-IRS combinations would generally be preferable mainly where LLINs are added onto IRS as opposed to where IRS is added onto LLINs. However, it must be realised that under programmatic circumstances, IRS cannot be expected to provide full year protection anyway. It is only sprayed periodically in selected areas, and usually not more than twice annually [22]. In other words, the practical limits of what can be expected from IRS under normal circumstances are much lower than the limits for LLINs. It is therefore very likely that in this thesis, the overall potential of IRS treatments may have been underestimated, given the apparent assumption that IRS should provide full year-round protection similar to bed nets. In epidemic situations for instance, IRS treatments can kill significant proportions of vector populations and therefore dramatically drive down malaria transmission at a geographical foci, at least on the short term. Moreover, where the public health systems are adequately organised and well funded enough to tackle the logistical challenges associated with repeated IRS campaigns, it is very likely that addition of IRS would significantly impact upon the vector population and malaria burden. Nevertheless, if we were to argue purely within the confines of our empirical data and theoretical simulations, overall community

level benefits would be more readily observed where LLINs are added onto IRS than where IRS are added onto LLINs. In both scenarios however, it is advisable that the selected IRS chemicals have high toxicity against target malaria vectors.

A key concern that has featured in this study is the poor performance of some of the most common vector control applications. For example, Olyset[®] nets which are currently the most common LLIN in Tanzania had extremely low toxicity and also low deterrence against the malaria vectors in both the two spray rounds (Chapter IV). Moreover, standard bioassay tests performed on this net showed that its toxicity against malaria vectors was significantly reduced after six months (Chapter V). It was therefore clear that any protection from Olyset[®] nets was mainly due to the physical barrier that it provides against mosquito bites, rather than its insecticidal properties. The products that we tested were obtained from the regular supply chain in-country and therefore represent the products that are actually being used by the target population. Given that that approximately 30 million of these nets are being produced every year in Tanzania alone [70], and also the fact that this is the most widely distributed LLIN in the region, its poor performance should be considered a major challenge and addressed promptly by the public health authorities.

Major limitations of the research

The research presented here, albeit being generally successful, was not without limitations. In this section I present the main limitations and suggestions of how to address them in future research.

The first limitation was in the experimental hut study presented in Chapter IV, which was conducted in two spray rounds. Here, we observed considerable variability in data obtained from the first spray round compared to the second round. For

example, whereas in the first round DDT elicited moderate levels of deterrence against the malaria vectors, this was not apparent during the second spray round. Also, the apparent increase in mosquito catches inside huts with pirimiphos-methyl alone or in combination with Icon Life® nets or PermaNet® nets, was more pronounced in the second round relative to the first round. It is unclear what the likely cause of these difference could be, given that the two spray round were not only conducted at different times but that the second spray round also incorporated a number of incremental improvements relative to the first round. For example, the first round consisted of fewer replicates (at least 40) than the second round (at least 60). Moreover, whereas in the second round, IRS huts had been randomly assigned, this had not been the case during the first round. It can therefore be argued that some of these differences could have been reduced or eliminated if the experiment had included more replicates and complete randomization in both spray rounds. We noted however, that more mosquitoes were caught during the second spray round than the second spray round, most likely because the second round happened during the wet season (November 2010 to April 2011), while the second round was in the dry season (April 2010-August 2010).

The second limitation regards the mathematical simulations presented here (Chapters VI-VIII), which were relied on a number parameter values obtained from a variety of sources, not necessarily representative of Africa-wide epidemiological scenario, and also a number of assumptions which may not necessarily proven in real life. These are common challenges in most mathematical models are must be considered when making inferences from results of any such simulations. Nevertheless, in the work presented here, significant attempts were made to ensure that all assumptions and parameter values incorporated in the simulations were

carefully evaluated and that they reasonably matched the desired epidemiological characteristics. Moreover, the key intervention parameter values used in the final modelling chapter (Chapter VIII), which describes community wide effects of LLIN/IRS combinations, were obtained from a single experimental hut study (Chapter IV). It is therefore not a surprise that results of these simulations generally mirrored those of the experimental hut study (Chapter IV).

There were also some limitations regarding the actual experimental designs. For example in the field experiments described in Chapters III and IV, the human volunteers sleeping in the experimental huts were not rotated, but were instead fixed to their hut locations. This was done to minimize logistical challenges associated with rotating the 18 volunteers over 9 experimental huts during the course of the study, a situation which would significantly increase variability in the data set. Instead, the variations associated with the human volunteers and those associated with the actual position of the experimental huts were lumped together and statistically considered as being a single source of variation. Moreover, in cases where the candidate intervention can itself be rotated, e.g. bed nets, volunteer rotation may sometime be unnecessary, but where the test intervention is fixed onto the huts and cannot possibly be rotated, e.g. IRS treatments, volunteer rotation becomes of considerable importance and should always be considered, where feasible [71]. It would therefore be more advisable that a smaller set of treatments are evaluated, with fewer huts, so that the human volunteers could be rotated alongside the actual candidate treatments.

Lastly, even though we had only 9 experimental huts available for this research, we included a considerably large number of treatments in our trial (i.e. 3 IRS insecticides and one unsprayed house, plus 4 LLIN types and an untreated net). This practice enabled us to assess combinations of a variety of insecticides classes currently

available for malaria vector control [37], with up to 16 different IRS-net combinations tested, but it also meant that the experimental design was weakened. For example, it was not possible to achieve the desirable number of replications until after six months, during which time the activity of the candidate applications had significantly decreased. Whereas it would have been more advisable to limit the number of insecticide applications tested, or to increase the number of experimental huts used, our approach was a reasonable trade-off considering the need to test multiple insecticide classes currently in use and the logistical challenges coupled with the cost constraints associated with constructing additional huts.

Whereas these methodological weaknesses and consequent observations may limit the strength of our findings, and therefore require more careful considerations, the findings on effects of combining LLINs with IRS are unlikely to be affected in any way that would affect our overall conclusions.

Summary recommendations and implications of the research findings for malaria control policy in Africa

At the early stages of this work, it was determined that there are numerous theoretical justifications for the application of IRS combined with LLINs. This research was to generate direct evidence to support or disprove these combinations. Due to the amount of resources and time available, the study could not test all the theories available. However, we have been able to gather evidence that is considerably relevant to some of the most common situations where LLINs and IRS are combined. It should be noted that the view adopted here is purely based on the research evidence and is not in anyway aimed at promoting any of the LLIN or IRS products. Give potential public health and economic implications of LLIN/IRS combinations for malaria vector

control in different places, these findings should be used with the full understanding of experimental and epidemiological circumstances under which our studies have been conducted. Even though detailed recommendations relevant to each of the aspects of this study are already included in the relevant chapters, the key issues are summarised into ten points here below:

1. Combinations of LLINs with IRS can be synergistic or redundant, depending on the types of insecticides used. Nonetheless, they would be most effective if any of the current LLINs are combined with highly toxic IRS treatments, one example being actellic.
2. Where people already have LLINs, addition of IRS would likely become redundant because: a) mosquitoes prevented from feeding may not rest indoors for long enough to pick up lethal IRS doses, and b) the rates of decay of commonly used IRS insecticides from sprayed surfaces, coupled with logistical challenges usually associated with re-spraying campaigns would make it impractical to maintain an all year round continuous coverage with effective IRS. Therefore, such an addition of IRS onto LLINs should be considered worthwhile only where adequate extra financial resources are guaranteed and where there are sufficient logistical mechanisms that would allow optimal IRS implementation. Even then, the selected IRS must be that which is highly toxic to mosquitoes.
3. Where IRS is the pre-existing intervention, addition of LLINs is mostly synergistic, and should be encouraged, particularly to provide direct personal

protection from mosquito bites, but also to provide continued protection when the activity of IRS has decayed. Where LLINs are considerably expensive or unavailable, then untreated nets should be considered as the basic minimum, so that IRS is never used alone.

4. Where resources are limited, priority should be given to providing everybody with LLINs and ensuring that these nets are consistently and appropriately used, and replaced at sufficiently frequent time intervals, rather than trying to implement both LLINs and IRS in the same community at the same time.
5. The use of long lasting untreated nets (LLUNs) that provide a robust and long lasting physical barrier against mosquito bites, and which can be used either in rotation with existing LLINs or in combination with current IRS insecticides should be debated and tested as a potential means for insecticide resistance management in malaria endemic communities in Africa.
6. Insecticides used in IRS and LLINs should be of different chemical classes, to generate maximum impact while at the same time minimising the risk of proliferation of insecticide resistance. Given that all existing LLINs are currently pyrethroid based, and because of possibilities of cross-resistance between DDT and pyrethroids, IRS with either DDT or pyrethroids should be discouraged in Africa.
7. It is important to always attempt to achieve high coverage with the LLIN and IRS interventions, so as to ensure that mosquitoes that fail to access blood

meals in intervention houses do not have nearby alternative human hosts who are unprotected. This is of particular importance in situations where interventions that deter significant proportions of mosquitoes, such as DDT or LLUNs are the only ones available for use. Even though household level efficacy of interventions can be very high where deterrent IRS is combined with LLINs, this outcome would translate to high communal protection only if: a) most of the households in the community are covered, b) the predominant vector species do not readily feed on other available alternative hosts such as cattle, nor readily bite people outdoors, and c) if people do not spend substantial amount of time outdoors at night or in the evenings.

8. Given the differences in experimental hut designs currently being used for testing indoor insecticidal interventions, attempts should be made to harmonise either the actual methodologies used to collect data, or the ways that the generated data is interpreted. In this regard, it is important to consider not only the insecticidal properties as classically described on the basis of toxicity, deterrence and irritancy, but also the actual protection that users or communities obtain even from the mere fact that some interventions such as untreated nets are also physical barriers against biting mosquitoes.
9. Efforts to identify new insecticides for use in LLINs and IRS should be enhanced to ensure a wide array of compounds for use in rotations, mosaics combinations or insecticide mixtures [20]. Availability of these options could allow many of the existing effective insecticides, notably pyrethroids to

continue to be used, for example in combinations consisting of pyrethroid IRS and non-pyrethroid LLINs.

10. Efforts to develop complementary interventions that are non-insecticidal and can be used outdoors should be enhanced as these would help deal with the extradomicillary residual transmission that continues to occur away from the direct reach of LLINs and IRS. This way the possibilities of closing the transmission control gaps and driving malaria towards its elimination will be enhanced.

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Cover sheets for chapters based on research papers

The following coversheets contain important details of all research papers included in this thesis. These details include:

1. Research paper Title
2. Publication status
3. Whether the work has been peer reviewed
4. Journal reference
5. Copyright declarations
6. Author names
7. Author contributions
8. Supervisor approval

No cover sheets are included for the non-research papers (i.e. Chapter 1: General Introduction and Chapter VIII: Discussion and Summary)

Cover Sheet for Chapter II

1. **Title:** Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future
2. **For a 'research paper' already published**
 - 2.1. **Where was the work published?** *Malaria Journal*
 - 2.2. **When was the work published?** *July 2011*
 - 2.2.1. **If the work was published prior to registration for your research degree, give a brief rationale for its inclusion**
N/A
 - 2.3. **Was the work subject to academic peer review?** *Yes*
 - 2.4. **Have you retained the copyright for the work?** *Yes*
If yes, attach evidence of retention: © 2011 Okumu and Moore; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. <http://www.malariajournal.com/content/10/1/208/abstract>
3. **For a 'research paper' prepared for publication but not yet published**
 - 3.1. **Where is the work intended to be published?**
N/A
 - 3.2. **List the paper's authors in the intended authorship order**
N/A
 - 3.3. **Stage of publication – Not yet submitted/Submitted/Undergoing revision from peer reviewers' comments/In press**
N/A
4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

I conducted the review and drafted the manuscript. Both my supervisor and I verified the information collected. I wrote the initial version, and my supervisor contributed to the final version of the manuscript

Cover Sheet for Chapter III

1. **Title:** A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: the Ifakara Experimental Huts
2. **For a 'research paper' already published**
 - 2.1. Where was the work published? PLoS ONE
 - 2.2. When was the work published? Feb 2012
 - 2.2.1. If the work was published prior to registration for your research degree, give a brief rationale for its inclusion
N/A
 - 2.3. Was the work subject to academic peer review?
N/A
 - 2.4. Have you retained the copyright for the work? N/A
If yes, attach evidence of retention: N/A
3. **For a 'research paper' prepared for publication but not yet published**
 - 3.1. Where is the work intended to be published? PLoS ONE
 - 3.2. List the paper's authors in the intended authorship order
Fredros O. Okumu' Jason Moore' Edgar Mbeyela, Mark Sherlock' Robert Sangusangu, Godfrey Ligamba, Tanya Russell, and Sarah J. Moore. _____
 - 3.3. Stage of publication –Published
4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

All the experiments were designed by me, my supervisor, Dr. Sarah Moore and Dr. Tanya Russell. I analysed the data under supervision of Dr. Moore and drafted the original manuscript for publication. My supervisor and I then edited the manuscript before submitting it to the journal. Jason Moore and my supervisor were involved in the initial designs of the experimental huts. Jason Moore was the lead engineer who constructed the huts and all its accessories including the traps and baffles. Finally, Edgar Mbeyela, Mark Sherlock, Robert Sangusangu, and Godfrey Ligamba supervised various field experiments described in this chapter.

Cover Sheet for Chapter IV

1. **Title:** Comparative evaluation of combinations of long lasting insecticidal nets and indoor residual spraying, relative to the use of either method alone, for malaria vector control in an area dominated by *Anopheles arabiensis*
2. **For a 'research paper' already published**
 - 2.1. **Where was the work published?** N/A
 - 2.2. **When was the work published?** N/A
 - 2.2.1. **If the work was published prior to registration for your research degree, give a brief rationale for its inclusion**
 N/A
 - 2.3. **Was the work subject to academic peer review?**
 N/A
 - 2.4. **Have you retained the copyright for the work?** N/A
If yes, attach evidence of retention: N/A
3. **For a 'research paper' prepared for publication but not yet published**
 - 3.1. **Where is the work intended to be published?**
 Malaria Journal
 - 3.2. **List the paper's authors in the intended authorship order**

Fredros Okumu, Edgar Mbeyela, Godfrey Ligamba, Jason Moore, Beatrice Sumaye, Lena Lorenz, Elizabeth Turner, Mike Kenwood, and Sarah J Moore.
 - 3.3. **Stage of publication** –Not yet submitted-
4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

All the experiments were designed by me with the assistance and supervision of Dr. Sarah Moore. Statistical training and support was provided to me chiefly by Dr. Lena Lorenz, but also by Dr. Liz Turner and Prof. Mike Kenwood, both at the LSHTM. I drafted the original manuscript, after which my supervisor and I edited it into a journal ready format. Edgar Mbeyela, Godfrey Ligamba assisted with the supervision of the field experiments. Jason Moore was the lead engineer in charge of field logistics, hut construction and maintenance. Finally, Dr. Beatrice Sumaye, conducted all the molecular analysis of mosquitoes, as reported in the chapter.

Cover Sheet for Chapter V

1. **Title:** Bio-efficacy and persistence of insecticides used for indoor residual spraying and long lasting insecticide nets in an area of weakening susceptibility among malaria vectors
2. **For a 'research paper' already published**
 - 2.1. **Where was the work published?** N/A
 - 2.2. **When was the work published?** N/A
 - 2.2.1. **If the work was published prior to registration for your research degree, give a brief rationale for its inclusion** N/A
 - 2.3. **Was the work subject to academic peer review?** N/A
 - 2.4. **Have you retained the copyright for the work?** N/A
If yes, attach evidence of retention: N/A
3. **For a 'research paper' prepared for publication but not yet published**
 - 3.1. **Where is the work intended to be published?**
 Malaria Journal
 - 3.2. **List the paper's authors in the intended authorship order**

Fredros Okumu, Edgar Mbeyela, Edith Madumla, Godfrey Ligamba, Jason Moore, Beatrice Chipwaza, and Sarah J Moore
 - 3.3. **Stage of publication** –Not yet submitted-
4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

All the experiments described here were designed by myself with assistance from my supervisor, Dr. Sarah Moore. I drafted the original manuscript, after which my supervisor and I edited it into a journal ready format. Edgar Mbeyela, Edith Madumla and Godfrey Ligamba assisted in performing the monthly bioassays and the susceptibility tests. Jason Moore was in charge of logistics and hut maintenance. Finally, Dr. Beatrice Sumaye conducted all the molecular analysis to detect insecticide resistance genes.

1. **Title:** Potential benefits, limitations and target product-profiles of odor-baited mosquito traps for malaria control in Africa
2. **For a ‘research paper’ already published**
 - 2.1. **Where was the work published?** PLoS ONE
 - 2.2. **When was the work published?** July 2010
 - 2.2.1. If the work was published prior to registration for your research degree, give a brief rationale for its inclusion N/A
 - 2.2.2. Was the work subject to academic peer review? Yes
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<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0011573>
3. **For a ‘research paper’ prepared for publication but not yet published**
 - 3.1. **Where is the work intended to be published?** N/A
 - 3.2. **List the paper’s authors in the intended authorship order** N/A
 - 3.3. **Stage of publication** N/A
4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

Though this research primarily represents my own work, I acknowledge that I was assisted by other researchers as follows: Dr. Nicodem J. Govella and Dr. Sarah J. Moore (my supervisor), assisted during original development and modifications of the model used here. Dr. Sarah Moore also supervised the sourcing and extraction of important intervention parameter values to be included in the simulations. Dr. Nakul Chitnis reviewed the model and the annotations to ensure mathematical consistency. Dr. Gerry F. Killeen taught me the basic ideas, and supervised the entire modelling exercise, and provided basic parameter values that were used in the basic simulations. I drafted the manuscript for publication, after which Dr. Sarah Moore and Dr Gerry Killeen then assisted in editing it prior to journal submission.

1. **Title:** Target product profile choices for intra-domiciliary malaria vector control pesticide products: repel or kill?

2. **For a 'research paper' already published**

2.1. **Where was the work published?** Malaria Journal

2.2. **When was the work published?** July 2011

2.2.1. **If the work was published prior to registration for your research degree, give a brief rationale for its inclusion**

N/A

2.3. **Was the work subject to academic peer review?** Yes

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<http://www.malariajournal.com/content/10/1/207/abstract>

3. **For a 'research paper' prepared for publication but not yet published**

3.1. **Where is the work intended to be published?** N/A

3.2. **List the paper's authors in the intended authorship order** N/A

3.3. **Stage of publication** N/A

4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

This was a co-authored chapter. However in this case, the research paper was not primarily my own work, but Dr. Gerry Killeen's. My participation was that I provided the basic modelling equations (from my work in Chapter VI) and participated in its improvements before new simulations could be done. I also acted as the last author of the published manuscript, ensuring consistency and authenticity of both the parameter values and the equations. Dr. Sarah J. Moore (my supervisor), and Dr. Nakul Chitnis participated by ensuring that the annotations and the parameter extractions were appropriately conducted. Dr. Killeen drafted the first manuscript, after which himself and I participated in writing the final version of the published manuscript.

1. **Title:** Simulated community-level effects of combining long lasting insecticidal nets with indoor residual spraying for malaria control in Africa

2. **For a ‘research paper’ already published**

2.1. **Where was the work published?** N/A

2.2. **When was the work published?** N/A

2.2.1. **If the work was published prior to registration for your research degree, give a brief rationale for its inclusion**

 N/A

2.3. **Was the work subject to academic peer review?** N/A

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If yes, attach evidence of retention: N/A

3. **For a ‘research paper’ prepared for publication but not yet published**

3.1. **Where is the work intended to be published?** Malaria
 Journal

3.2. **List the paper’s authors in the intended authorship order**
 Fredros O. Okumu, Sarah J. Moore, and Gerry F. Killeen

3.3. **Stage of publication** N/A

4. **For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)**

Though this was also a co-authored research paper, the work included here was primarily my own. All the simulations described here were performed by me, with the assistance and supervision of Dr. Gerry Killeen. I drafted the original manuscript, after which I worked together with Dr. Gerry Killeen and Dr. Sarah Moore, to finalize it into a journal ready format.